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Influence of Autonomic Nervous Centers on Liver Glyconeogenesis

By

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The experiment of the diabetic puncture by Cl. Bernard made it known that the autonomic nervous centers lying in the hypothalamus have a carbohydrate metabolism regulating function. Following this Eckhard[1] and Miyake[2] clarified the existence of the centers for glycosuria and water metabolism at the bottom of the fourth ventricle, Aschner[3] at the bottom of the third ventricle, and Leschke and Schneider[4] at the hypothalamus. Brugsch, Dressel and Lewy[5] stated that there is at the corpus striatum a higher center which regulates the hypothalamic centers and that a part of vagus nerve nucleus in the medulla oblongata are sympathic nerves and centers of carbohydrate metabolism. Other reports were published by Watari and Ochi,[6] Maeda,[7] Sunaba,[8] Ishijima,[9] Mizuta,[10] Shinozaki[11] on this subject. Kurotsu[12] has published recently a remarkable theory.

According to his opinion there are autonomic nervous centers in the hypothalamus at the bottom of the third ventricle. He devided the hypothalamus into three cell regions, naming them a parasympathic, b sympathic and c parasympathic zone respectively towards the outside of the third ventricle wall. Shimizu[13], his co-worker, reported that b sympathic zone puncture caused hyperglycaemia and that of c parasympathic zone hypoglycaemia. Yamada[14] acknowledged that b sympathic zone puncture stimulates bile secretion by liver cells, while that of c parasympathic zone checks this secretion.

The question of carbohydrate metabolism can not be fully discussed merely from the point of blood sugar, naturally it must go further to the relation with glyconeogenesis and glycolysis. However, we have not had any reports on this research.

My intention of trying these experiments was to more clarify the relation between the glyconeogenesis function of the liver and autonomic nervous centers.

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Experimental

Methods

For my experiments I used adult male rabbits which had been kept for about 10 days with no other food given than 'tofukara.' For 24 hours just before the experiment they had been given nothing to eat. Methods employed were that of Kurotsu[12] for autonomic nervous center puncture and the micromethod by Kurokawa and others[15] for liver glycogen estimation.

A 25 g/dl solution of glucose for injection was the glycogen material, 10 cc. of which was administered to the rabbits intravenously for each kg. of their weight.

Results were obtained by arithmetrical average of two rabbits for each experiment.

Results

1. Control test

As control test laparotomy was performed upon the rabbits which had been in hungry state for 24 hours without any administration given to them. Immediately after the operation and 1, 2, 3, 4, and 5 hours after it about 10 mg. of liver specimen were drawn out for the examination of how the liver glycogen content changed.

Fluctuation range of liver glycogen content lay between −0.021% and +0.078% till 5 hours after the laparotomy, not showing remarkable differences (Table I).

Table II is the result of another control test where 10 cc. of 25 g/dl glucose solution per kg. was intravenously injected into the rabbits. Here I can see that liver glycogen content culminated 3 hours after the laparotomy, indicating an increase of 1.606% compared with that shown im-

Table I
Control Test

No. of rabbit	Date	Body weight (g)	Room temperature(°C)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
1	22/I	1650	2	4	0.240	0.175	0.230	0.263	0.238	0.236
2	16/IV	1980	9	17	0.302	0.326	0.416	0.436	0.437	0.301
Average			5.5	10.5	0.271	0.250	0.323	0.349	0.337	0.268
	Difference from immediately after					−0.021	0.052	0.078	0.066	−0.003

TABLE II

Glucose Injection Test in Jan. and Feb.

No. of rabbit	Date	Body weight (g)	Room temperature (C°)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
3	9/I	1970	0	4	0.874	1.579	2.069	2.341	2.567	2.579
4	18/I	1900	−1	4	0.716	1.786	2.371	3.273	3.312	3.310
5	20/I	1900	2	4	0.187	0.842	0.695	0.872	0.750	0.518
6	1/II	2430	−1	2	0.651	1.868	2.435	2.594	2.556	2.362
7	5/II	1650	−2	2	0.352	1.133	1.721	1.822	1.790	1.266
8	10/II	1800	1	3	1.306	2.288	2.371	2.825	2.604	2.318
Average			−0.1	3.1	0.681	1.582	1.943	2.287	2.263	2.058
	Difference from immediately after					0.901	1.262	1.606	1.582	1.377

mediately after it.

2. b sympathic zone puncture

1) b sympathic zone puncture and simple laparotomy test

When b sympathic zone was punctured without glucose administration, liver glycogen content showed a decrease of 0.082% an hour after the puncture in comparison with that measured immediately after the laparotomy; and further, two hours after it showed a decrease of 0.600%, presenting the lowest figure. After 3 hours, 4 hours and even 5 hours respectively decreases were 0.332%, 0.032%, and 0.430% (Table III).

TABLE III

b Sympathic Zone Puncture and the Simple Laparotomy

No. of rabbit	Date	Body weight (g)	Room temperature (°C)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
9	9/II	2080	1	3	2.067	1.962	1.675	1.762	2.320	1.778
10	14/II	2130	−1	2	1.011	0.955	0.204	0.652	0.693	0.440
Auerage			0	2.5	1.539	1.458	0.939	1.207	1.506	1.109
	Difference from immediately after					−0.082	−0.600	−0.332	−0.032	−0.430

Compared with the contents examined in control tests the decreases observed here were very remarkable.

2) b sympathic zone puncture and the glucose injection

10 minutes after the b sympathic zone puncture glucose solution was injected into the vein and the liver glycogen was measured. The results obtained were tabulated as Table IV.

TABLE IV

b Sympathic Zone Puncture and the Glucose Injection

No. of rabbit	Date	Body weight (g)	Room temperature (°C)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
11	13/II	1700	0	4	0.790	0.567	0.284	0.700	1.372	1.352
12	29/II	1660	1	3	3.021	2.779	4.062	3.741	3.862	4.369
Average			0.5	3.5	1.905	1.673	2.173	2.220	2.617	2.860
	Difference from immediately after					−0.232	0.268	0.315	0.712	0.955

The liver glycogen content measured an hour after the b sympathic zone puncture decreased 0.232%, though glucose had been given, compared with that soon after the laparotomy. After two hours the content only showed an increase of 0.268%. After 3 hours the increase was 0.315%, after 4 hours 0.712% and after 5 hours 0.955%. This increase compared with the highest value of increase in control test, 1.606%, it was acknowledged that the ability of liver for glyconeogenesis was here considerably checked.

3. c parasympathic zone puncture test

1) c parasympathic zone puncture and the simple laparotomy

As is shown in Table V, the increase of liver glycogen content when puncturing was merely made in c parasympathic zone, was 0.758% an hour after the treatment compared with the content just after the lapraotomy was performed. The glycogen content increased 0.905% two hours after and 1.075%, the highest value, three hours after. The increases became 0.977% and 0.862% respectively after four and five hours of the puncture. Marked increases of liver glycogen content were observed compared with the case of control test.

2) c parasympathic zone puncture and the glucose injection

When glucose solution was intravenously injected ten minutes after the puncture in c parasympathic zone, the measured liver glycogen contents furnished us with Table VI. An hour after the c parasympathic zone puncture liver glycogen content increased 1.433% compared with that immediately after the laparotomy. The increase grew 2.292% after two hours and 2.686% after three hours giving the highest figure of per-

TABLE V

c Parasympathic Zone puncture and the Simple Laparotomy

No. of rabbit	Date	Body weight (g)	Room temperature (°C)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
13	26/I	2070	2	4	0.993	1.634	1.959	2.381	2.217	2.037
14	30/I	2070	1	3	1.591	2.466	2.435	2.354	2.322	2.272
Average			1.5	3.5	1.292	2.050	2.197	2.367	2.269	2.154
	Difference from immediately after					0.758	0.905	1.075	0.977	0.862

TABLE VI

c Parasympathic Zone Puncture and the Glucose Injection

No. of rabbit	Date	Body weight (g)	Room temperature (°C)		Liver glycogen content (%)					
			cote	laboratory	immediately after	1h	2h	3h	4h	5h
15	25/I	2000	2	4	0.567	2.241	2.855	3.313	3.443	3.486
16	19/II	2650	−1	2	1.357	2.550	3.653	3.984	3.804	3.197
Average			0.5	3	0.962	2.395	3.254	3.648	3.623	3.341
	Difference from immediately after					1.433	2.292	2.686	2.661	2.379

centage. After four hours it became 2.661%, then after five hours 2.379%. This value of 2.686% shows, when compared with the highest 1.606% in the control test which was obtained after three hours, a remarkable increase of liver glycogen content, an enormously activated function of the liver for glyconeogenesis.

DISCUSSION

As to the influences of autonomic nerves on the liver function Kawakatsu[16] stated that sympathic nerves checked the excretion of injected dyes from the liver, while parasympathic nerves on the contrary quickened it. Nariyama's[17] report was that the puncture of diencephalon, especially nucleus paraventricularis, caused an immense excitement of this function of the liver.

Yamagata, Aratani and Miura[18] from our Clinic, on the contrary, observed that after b sympathic zone puncture the congo-red index was lowered and blood prothrombin index elevated, and c parasympathic zone puncture induced contrariwise the reverse phenomenon, suggesting

the close bearing of autonomic nervous centers on the function of the liver.

On the relation between autonomic nerves and liver glycogen the same result was reported by Drumond and Paton[19], Doyon and Kareff[20], Agadschanianz[21], Olmsted and Coulthard[22], Sato[23], Oikawa[24] that liver glycogen content was made to lessen by injection of adrenalin, a sympathic nerve poison. But no report has been made on the condition of liver glycogen content after direct stimulus has been given to autonomic nervous centers.

After Kurotsu's[12] method I tried autonomic nervous center puncture and obtained the following result: when merely stimulus was given to b sympathic zone without injecting any glycogen materials, liver glycogen content was distinctly decreased compared with the case of control test and when stimulus was given to c parasympathic zone, liver glycogen content increased remarkably in comparison with the control test. But when an intravenous injection of glucose was made as a glycogen material, the stimulated b sympathic zone restrained the glycogen producing ability of the liver, while the stimulated c parasympathic zone activated the function to a remarkable degree.

The results of my experiments that puncture in b sympathic zone hampered markedly the faculty of glyconeogenesis and that of c parasympathic zone made it markedly active correspond to the reports made by Yamagata, Aratani and Miura[18], and coincide with Shimizu's[13] statement that when he punctured b sympathic zone blood sugar increased and it decreased in case of b parasympathic zone puncture.

It may safely be said that my experimental results have clarified the relation between autonomic nervous centers and the liver function of glyconeogenesis.

Conclusion

I tried autonomic nervous center puncture by adopting Kurotsu's[12] method and arrived at the following conclusions.

1. There is a close correlation between liver glycogen content and autonomic nervous centers.

2. When b sympathic zone is punctured liver glycogen content decreases and also when glucose as a glycogen material is administered the glyconeogenesis function of the liver is markedly restrained.

3. When c parasympathic zone is punctured liver glycogen content increases and also when glucose is injected as a glycogen material the glyconeogenesis function of the liver is noteworthily activated.

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On the Z-Granules in the Granulocyte Series, Preliminary Report

By

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Yamasaki[1] pointed out the existence of a new kind of granule, generally characteristic to the neutrophile and pseudoeosinophile granulocyte series, and called this element ζ (Z) granule which follows α-ε granules already known. Recently we have noticed that the Z-granules are metachromatically stained with various basic dyes and this stainability becomes evident and remarkable in the coexistence of sodium salts.

The granules may be demonstrated most constantly by the following technique:

Material: Imprint or smear of the bone marrow and smear of the peripheral blood.

Procedure: 1) Fix the specimens in methanol, 2) stain in aqueous solution of thionin (0.2%) and sodium acetate (5%) and then 3) wash in the water.

Result: Z-granules are stained yellow, reddish yellow to brown among the special granules which are not stained.

The distribution and quantity of the Z-granules in each of the granulocyte series is shown in Table I.

TABLE I

	Promyelocytes (mature……immature)			Myelocytes	Metamyelocytes	Polymorphonuclears		
	bone marrow			〃	〃	〃	streaming blood	inflammatory area
Rabbit	+	++	+++	+++	++	+	(+)	+++
Human	(+)	+	++	++	+	(+)	±	( )

+++ most numerous, ++ numerous, + moderately numerous
(+) few, ± rare, ( ) yet unknown.

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Relation of the Parotid Gland to Serum Protein and Malnutrition

By

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Enlargement of the parotid gland which has heretofore been observed mainly in the patients of diabetes mellitus (Flaum,[1] John,[2] Takaoka,[3]) thyroid diseases (Haemmerli[4]) and obesity (Sprinzels[5]), became conspicuously noticeable among malnourished people especially after the World War II. In Japan swellings of parotid region have been reported first by S. Katsunuma[6] in the repatriated Japanese war prisoners. And our medical colleagues repatriated from Soviet war prisoner camps in Siberia informed us that many Japanese war prisoners in the said district suffered from epidemia of mumps, which showed no sign of fever, redness and pain.

Davies[7] stated that a very first sign of clinical improvement from a malignant malnutrition, so-called 'Kwashiorkor' found commonly among the African natives is a swelling of the parotid and other salivary glands as a curious finding. Great Britain Medical Research Council[8] noticed an enlargement of the parotid gland among German malnourished war prisoners repatriated from Soviet Russia, and their parotid glands enlarged histologically to be hypertrophy of acinus cells. However, it should be pointed out that no one has yet offered a satisfactory explanation on the cause of these enlarged parotid glands.

We[3] have found since 1946, by our palpating method of the parotid gland that over 80% of 150 diabetics have a swelling in their parotid glands and moreover, that the said swelling varies in parallel with a clinical condition of the diabetics. While we have proved that extracts from the salivary glands of alloxan diabetic dogs reduced the blood sugar levels of normal rabbits, we have advocated that the salivary gland participates in the carbohydrate metabolism as endocrine organ, and plays a compensatory role of the pancreas in the diabetes mellitus.

Although alloxan diabetic dogs with the salivary glands extirpated showed a reduction of fasting blood sugar level and became sensitive to

insulin, clinical condition did not improve and became emaciated like malnutrition instead. To explain this contradiction we have conducted this research as follows in an attempt to confirm that whether salivary gland might possibly have an effect on protein metabolism as well as on carbohydrate metabolism or not.

Experimental

Methods

Dogs and rabbits were fed with a certain standard diet. Special attention was paid to macroskopic changes of their stools during the experiment. Total serum protein was examined with refractometer and serum albumin globulin ratio (A/G) with electrophotometer which was analysed by separating method of Campbell and Hanna[9] and by the application of biuret method of Weichselbaum.[10] Blood sugar estimation was determined by Hagedorn-Jensen's method and serum calcium by Sobel's method.

Parotin, an extract of parotid gland of cattle was prepared by A. Ogata[11] to reduce serum calcium based upon the T. Ogata's theory[12] concerning the internal secretion of the salivary gland on the skeletal metabolism. This sample is now being producted by Y. Ito, A. Ogata's successor, as pure protein and reduces about 12% of serum calcium of normal rabbits by the injection of 1 mg. per kg. of body weight.

Results

I. Removal of salivary glands of normal animals

Various tests have been conducted on five dogs and five rabbits for the period of three months after extirpating all of their salivary glands. During this period these animals showed no marked disturbances of mastication, nor any noticeable change in body weight, total serum protein, sugar tolerance curve and macroskopic examination of stools. But the serum A/G decreased markedly, in some extreme case approximately to half (Fig. 1). We could not recognize such changes of A/G in the two dogs with external fistulas of salivary glands.

II. Injection of Parotin to rabbits

If these results were due to the disturbance of internal secretion of the salivary glands, effective factor for the serum protein should be extracted from the salivary gland. The parotin mentioned above was first used in this experiment.

(1) As a control test total serum protein and A/G of five normal rabbits were examined before and 3, 8, 24 hours after injection of 1 cc.

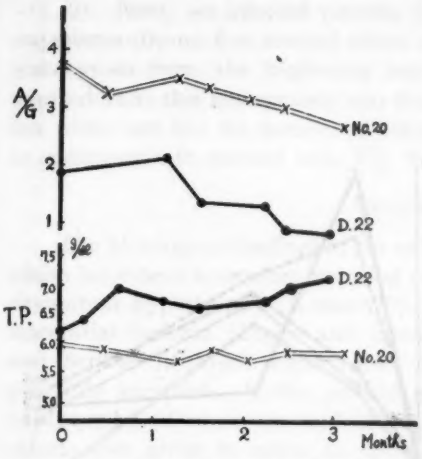

Fig. 1. Influence of removal of the salivary glands on total serum protein and A/G in normal dog and rabbit.

Dog
Rabbit

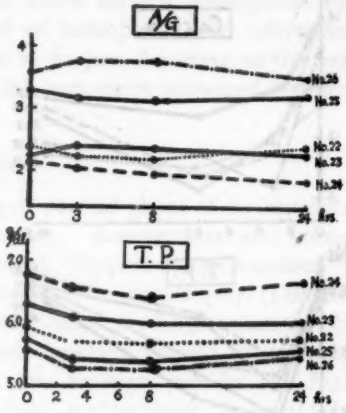

Fig. 2. Control test of total serum protein and A/G in five normal rabbits.

physiologic saline solution per kg. of body weight. The total serum protein varied within 0.4 g. and A/G showed no significant change (Fig. 2).

(2) We injected 10 mg. of parotin in 1 cc. of physiologic saline solution per kg. of body weight intravenously on 4 rabbits with the salivary glands removed. Three hours after the injection the total serum protein decreased as strongly as serum calcium and vice versa A/G increased considerably. This tendency of serum changes continued 8 hours after the injection and it was due to the reduction of globulin fraction.

(3) Next, we did the same on five normal rabbits and obtained exactly the same results (Fig. 3, 4). The change of A/G is now being investigated by us with an apparatus of the Tiselius electrophoresis.

It is clear from our experiments that the removal of salivary glands led to the reduction of A/G, and also parotin causes a serum A/G to be higher.

III. Influence of parotin on hemogramm

(1) We injected the same doses of parotin to examine the hemogramm on five normal rabbits, which showed marked leukopenia due to lymphopenia from two to three hours after the injection and considerable leukocytosis due to neutrophilia after the succeeding three to five hours

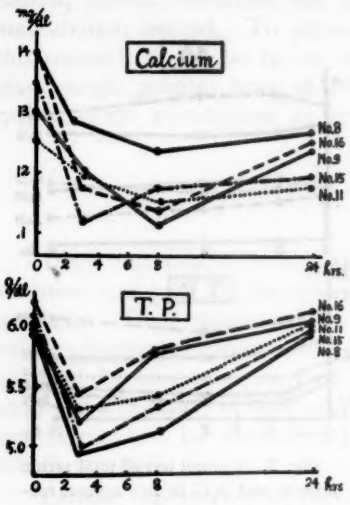

Fig. 3. Influence of the parotin on serum calcium and total protein in five normal rabbits.

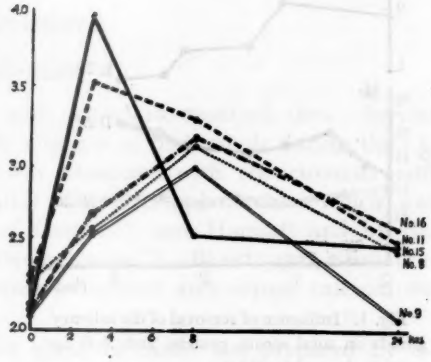

Fig. 4. Influence of the parotin on serum A/G in five normal rabbits.

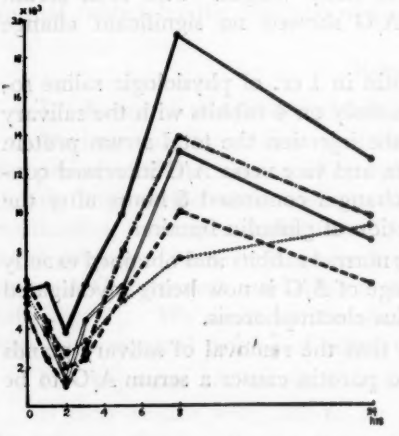

Fig. 5. Influence of the parotin on the number of circulating leukocytes in five normal rabbits.

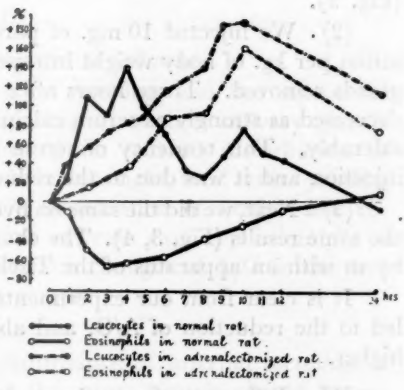

Fig. 6. Changes in the number of circulating leukocytes and eosinophils in normal and adrenalectomized rats.

until up to 24 hours (Fig. 5), but no change has occurred in number of erythrocytes, hemoglobin content and blood sugar level.

(2) Next, we injected parotin 3 mg. per 100 g. of body weight intraperitoneally on five normal white rats, which showed eosinopenia and leukocytosis from the beginning instead of leukopenia. In adrenalectomized rats, this leukocytosis was found to be just the same as the said five white rats but the number of eosinophil leukocytes increased contrary to eosinopenia in normal rats (Fig. 6).

Discussion

The histological findings of the enlarged parotid gland of malnutrition which have been heretofore discussed are many. Among them are: parenchymatous hypertrophy (Kenawy[13]), " work " hypertrophy (Kanther[14]), interstitial oedema (Eigler and Boenninghaus[15]), or atrophy (Luckner and Scriba[16]). Work hypertrophy among those findings seems to be generally accepted. If the parotid gland, however, would cause work hypertrophy of mere external secretion due to high protein and high caloric diet given in order to recover from malnutrition, it would be possible to explain the parotid enlargement in case report of Davies thereby, but difficult to explain the parotid enlargement of greatly undernourished patients observed by Kenawy and Katsunuma.

The parotid enlargement of malnutrition, however, could very clearly, we believe, be explained by coordinating our experimental results on serum protein and palpating data of parotid gland of diabetics, although we have no opportunity at present of observing a malnourished people.

By our observation of diabetics for long time as reported previously, the parotid glands are remarkably swollen and histologically hypertrophic in acinus cells and " Streifenstueck " and contain blood sugar reducing factor mentioned above in improved condition of the main disease. But the parotid gland will become unpalpable or histologically atrophic in " Streifenstueck " in serious condition of the disease, and moreover even in additional acinus cells in the case of coma diabeticum and lose blood sugar reducing factor. As these phenomena are difficult to be explained simply by the amount of food intake, our thinking with respect thereto is as follows:

According to the theory of internal secretion of salivary gland by T. Ogata, the saliva is absorbed in " Streifenstueck " and acts as a hormone after entering into body through the way of lymph around the " Streifenstueck." We examined histologically parotid glands of 100 patients died of various diseases by autopsy in the Department of Pathology of this University and were convinced that the " Streifenstueck " is just as important and variegated as acinus cells. If the " Streifenstueck " is so important as T. Ogata asserts, it will be reasonable that the parotid gland with hypertrophied " Streifenstueck " has favourable influence on sugar

metabolism but the gland with atrophied "Streifenstueck" has not.

We wish to explain the enlarged parotid of malnutrition by the application of this clinical observation of diabetics in addition to our experiments on serum protein. If the parotid gland participates in protein metabolism as hormonal organ, the parotid would become atrophied parallel with the general atrophy of all other organs in acute serious malnutrition (case of Luckner), but could remain hypertrophied in not so serious malnutrition. Such undernourished patients with the parotid hypertrophied could recover possibly from the disease, even if their appearance seems to be seriously ill (cases of Takizawa[17] and Kanther). As the same individual becomes better due to general improvement in the diet, not only the external but also internal secretion of the parotid gland will become functionally more active and histologically hypertrophied (cases of Kenawy, McCance and Davies).

Finally, the correlation between the parotid gland and leukocyte should be discussed. The mechanism of parotin action on circulating leukocytes is difficult to be explained merely by permeability of the blood vessel or the reaction of adrenal cortex as general adaptation syndrom. After the end of last World War Takaoka advocated a swelling of parotid gland as a simple hypertrophy of the gland, cause of which could not be disclosed as like diabetes, thyroid disease, etc. Such individuals showed relative lymphocytosis of circulating blood. These facts give us the impression, as if the parotid gland has an intimate relation to circulating leukocytes.

It is reasonable, we believe, to conclude that the parotid gland as hormonal organ has relation to serum protein but it remains for future work to decide that the parotid gland participates in protein metabolism itself.

Summary

1. The serum A/G decreases after the removal of the parotid glands in dogs and rabbits.

2. The injection of parotin in doses of 10 mg. per kg. of body weight raises the serum A/G of normal rabbits, but does not change the blood sugar level. We assert, therefore, that the parotid gland participates in serum protein.

3. We discussed the possibility that the cause of parotid enlargement of malnutrition could be explained by our experiments.

4. Parotin causes leukopenia of normal rabbits on the beginning of injection and thereafter marked leukocytosis.

5. Parotin action of leukocytosis in normal white rats does not depend upon the existence of their adrenal cortexes.

We would like to express best thanks to Dr. Y. Takahashi for the technical advise in the serum A/G determination and to Drs. Akira Ikushima and H. Momoi for the examination with the Tiselius electrophoresis.

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On the Chemical Properties of the Z-Granules

I. Ribonuclease Test

By

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The existence of a new element, Z-Granule, in the granulocyte series which is demonstrable by a new staining method has been mentioned already by us.[1] A further evidence of the argument could be offered by ribonuclease test in a manner below.

The imprints of rabbit bone marrow are fixed in methanol for 3 minutes or in susa solution for 1 minute, then washed in the water. After incubation in buffered ribonuclease solution for 1–2 hours at 30°C, the specimens are rinsed with distilled water and stained with thionin- sodium acetate solution.[1] The enzyme solution is prepared by dissolving 5 mg. of ribonuclease in 10 cc of sodium phosphate-citric acid buffer (pH 5.6). The basophilia in the cells which represents ribonucleic acid is removed by the enzyme, while the Z-granules remains almost intact and is stained metachromatically with thionin, as when processed without prior enzymic digestion.

On the other hand, the application of Lugor and sodium thiosulfate following susa fixation enfeebled remarkably the stainability of the granules. Further to add, the granules are demonstrated under certain conditions in a colour similar to the azur granules by May-Giemsa stain.

The results obtained are illustrated in Table I.

TABLE I

		Fixed w. methanol		Fixed w. Susa			
		no treatment w. Lugor and sodium thiosulfate		no treatment w. Lugor and sodium thiosulfate		treated w. Lugor and sodium thiosulfate	
		digested w. ribo-nuclease	not digested	digested	not digested	digested	not digested
Stainability of Z-granules	w. thionin-sodium acetate	+	+	+	+	±	±
	w. May-Giemsa	−	−	+	+	±	−

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Rotatory Crossgraphy (Rotatory Cross Section Radiography) of the Neck

(Studies on Rotatography. 8th Report)

By

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(Received for publication July 2, 1952)

The apparatus[1)2)3)4)] employed for the present study of rotatory crossgraphy was the same as was described in the report published in a preceding number of this Journal. The exposure here continued 9 seconds, under the conditions of 57 kv of the tube terminal, 40 ma in the tube current, with the focus-body rotation table distance of 98 cm. and the body table-film table distance of 50 cm.

Results

23 healthy adults were roentgenographed at nine cross sections of the neck. Each of the sections was selected so that it might be horizontal to the base cross section which included the hyoid bone in the erect position. They are called in the order from top to bottom the First, the Second—and the Ninth Cross Section. The x-ray image of every cross section consists of three parts different in density respectively caused by the moderate opacity of soft tissue, by the opacity of bone, and by the translucency of the upper air passage.

First, in order to show the topographical features of the upper air passage and the cervical spine, the Second and the Fifth Cross Sections will be detailed below.

The Second Cross Section (The Base one) (Fig. not given). This was radiographed at the height of the hyoid bone.

The contour of the image is a deformed oval with its dull pole directed backwards. The hyoid bone, being convex at the front, is imaged most

anterior in the cross section; and the upper air passage, shaped trapezoid in the cross section, is situated at the back of this bone. Epiglottis, crooked occasionally, and plica pharyngoepiglottica which connects epiglottis and the lateral wall of pharynx, form a border line in pharynx, and divide the trapezoid of pharynx into two parts, namely, vallecula glossoepiglotica at the front and aditus laryngis at the back. The cervical spine is imaged at the center of the cross section. The canal of this spine is bordered anteriorly by a spinal body (C_4), laterally by arcus vertebrae and posteriorly by processus spinalis. At the back of this processus spinalis (C_4) is traversed the lowest part of processus spinalis (C_3), which is imaged as two separate pieces.

The Fifth Cross Section (Fig. 1). This was radiographed at the height of incisura thyreoidea cranialis, which is approximately 15 millimeter lower

Fig. 1. Crossgram of the neck. The Fifth cross section crossgraphed at the height of incisura thyreoidea in the erect position.

than the Base. This section is a little larger in size than the Second. The contour forms a deformed hexagon, which is due to the necks bending backwards at the time of roentgenography. Most anterior in the cross section is rima vestibulae, imaged pyramidal in shape, with the apex directed frontwards; and posterolateral to that and on each side is a pair of recessus piriformis, pyramidal and with its apex directed laterally. Rima vestibulae and a pair of recessus piriformis form an isosceles triangle, each being situated at one of its vertices. The cervical spine (C_5) is traversed at the back (about 5 mm.) of the upper air passage. A gap between the body and the arch of the spine corresponds to incisura vertebrae. A fleck at the back of processus spinalis (C_5) is the image of the forkshaped termination of processus spinalis (C_4).

The general view of the upper air passage in the cross section will be detailed then.

The Cross Section of Larynx (Fig. 2 and 3.) In the middle of pharynx, 5 mm. higher than the Base Cross Section, epiglottis is imaged with an

Fig. 2. Cross Section Radiograms of the upper air passage of the neck. The number of each cross section showing the order of traversing.

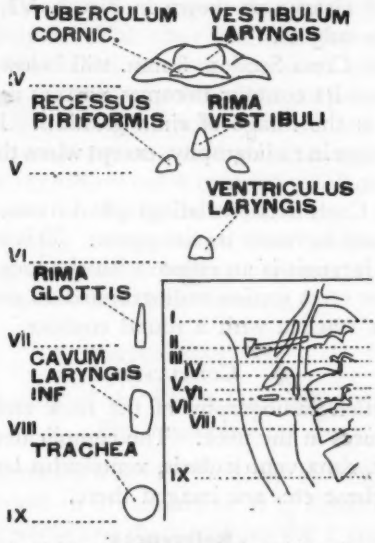

Fig. 3. Sketch of Fig. 3. Crossgraphed position shown at the right bottom.

arch, convex frontwards and dividing pharynx into two areas, that is, the area of the root of the tongue at the front and the opening of aditus

laryngis at the back. Projections from both of the lateral walls of pharynx are the images of plica palatopharyngea.

The x-ray image of the next cross section, namely, the Base one is detailed upper. Epiglottis increases its thickness and curvature, and vallecula formed by epiglottis and the root of the tongue at its front.

In the Third Cross Section radiographed 5 mm lower than the Second, vallecula is missed, and aditus laryngis, of which lateral wall is bordered by plica aryepiglottica, appears with the shape of an ellipse.

In the Fourth Cross Section, 5 mm. lower still, tuberculum corniculatum is imaged as a back border in the contour of this area of larynx. It looks like a butterfly spreading its wings. Vestibulum laryngis, which is bordered by two thin membranes of plica aryepiglottica, is imaged at the front of the wings of this butterfly.

The x-ray image in the next cross section is given in Fig. 1. This was radiographed only under the condition of normal breathing. Rima vestibularis and recessus piriformis are situated at the vertices of an isosceles triangle.

In the Sixth one raidographed 5 mm. lower, recessus piriformis has disappeared. Ventriculus laryngis is imaged indistinctly here. This, however, is imaged clearly, as shown in Fig. 3. VI, only when "A" is pronounced by the subject.

In the Seventh Cross Section, 3 mm. still below, ventriculus laryngis reduces its size, and its contour becomes narrow again on either of the both sides. This is the image of rima glottidis. Rima glottidis is difficult to gain an image in radiography, except when the subject is breathing normally.

In the Eighth Cross Section radiographed 5 mm below, rima glottidis changes in shape and increases its size again. This is the image of cavum laryngis. Cavum laryngis is an ellipse with the longitudinal axis directed frontwards. In the cross section radiographed 15 mm below still appears the x-ray image of trachea with a round contour.

Conclusion

The cross section radiography of the neck enables us to know the topographical features in the neck. The complicated structures of larynx and spine, namely, rima ventricularis, ventriculus laryngis, rima glottidis, and incisura vertebrae etc. are imaged there.

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Chemistry and Biology of Lipids

XIII. The Group Lipoid of Pig Pancreas

By

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(Received for publication, June 15, 1952)

14 fresh pig pancreases were separately stood with cold alcohol and the dehydrated materials treated with hot alcohol. The substances extracted with cold alcohol, the ones, precipitating and non-precipitating from the hot-alcoholic extracts when cooled, and the tissue residues were examined regarding the blood group activity. The third fractions all specifically inhibited the isoagglutination of **A** erythrocytes, whereas, of the first fractions, some could be recognized to effect the same action but some not due to hemolysis. The other fractions proved inactive. Next, the balance of the third fractions were combined and subjected to the procedure in preparing the group lipoids of human pancreases[1] with minor modifications. The divisions were tested on the sero-reactivity during the whole course, because between two kinds of animal, the corresponding substances of the same organs are not warranted to fall on the corresponding divisions in fractionation of the lipids in particular.

An electrophoretically homogeneous final product was much less active than the Group **A** lipoid of human pancreas and further diverged from the latter in composition to some extent.

Experimental

Examination of the Blood Group Activity of Pig Pancreases

14 fresh pig pancreases weighing from 42 to 115 g. were separately masticated and preserved in 3 volumes of 91% denatured alcohol. 5 of them were stood for 6 weeks and the remaining for 1 year. The materials were then collected on a funnel and after drying in a Faust apparatus, boiled twice with 5 volumes of the denatured alcohol for 2 hours. The successive extracts of each material, filtered while hot, were combined, placed in an ice chest for 1 day, whereby a precipitate (**II**) occurred. The

TABLE I

Weight of the Fractions of Pig Pancreases and Inhibition of Isoagglutination of Erythrocytes

—: group-inactive, +: Group **A** active, ±: probable Group **A** activeness.

Pig pancreas		I* washed with acetone		Hot-alcoholic extract				Tissue residue after hot-alcoholic extraction	
				II† washed with acetone		III‡ washed with acetone			
No.	wt. (g.)	wt. (g.)	group-activity	wt. (g.)	group-activity	wt. (g.)	group-activity	wt. (g.)	group-activity
1	46	1.4	+	0.02	—	0.5	+	4.0	—
2	55	1.1	±	0.06	—	0.6	+	3.0	—
3	115	2.6	+	0.09	—	1.0	+	8.7	—
4	78	1.1	±	0.27	—	1.1	+	6.4	—
5	98	3.1	+	0.09	—	1.0	+	6.9	—
6	81			0.04	—	0.6	+	6.0	—
7	64			0.18		0.8	+	3.2	
8	94			0.09		0.9	+	6.2	
9	42			0.02		0.5	+	2.6	
10	92			0.18		0.8	+	7.7	
11	38			0.01		0.4	+	4.2	
12	71			0.09		0.6	+	2.9	
13	96			0.18		1.4	+	9.2	
14	59			0.05		0.3	+	2.1	

* The fraction extracted with cold alcohol. † The precipitate from the hot-alcoholic extract on cooling. ‡ The substance that had been recovered by distillation of the hot-alcoholic extract after cooling and removing of the precipitate.

TABLE II

Occurrence of Isohemoagglutination, when Fr.-I's,* washed with Acetone, had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively.

—: no agglutination, ±: faint aggl., +: slight aggl., H: hemolysis.

Pig pancreas No.	Group of red cells	Dilution of substances				
		$1:10^2$	$1:2\times10^2$	$1:4\times10^2$	$1:8\times10^2$	$1:1.6\times10^3$
1	A	H	H	H	H†, —	+
	B	H	H	H†, +	+	+
2	A	H	H	H	H†, ±	+
	B	H	H	H	H†, +	H†, +
3	A	H	H	H	H†, —	+
	B	H	H	H†, +	+	+
4	A	H	H	H	H	H†, ±
	B	H	H	H	H†, +	+
5	A	H	H	H	H†, —	H†, ±
	B	H	H	H	H†, +	+

* Signifies the same as in Table I. † Hemolysis was partial.

Table III

Occurrence of Isohemoagglutination, when Fr.-III's,* washed with Acetone, had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively.
—: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., +++: complete aggl.

Pig pancreas No.	Group of red cells	Dilution of substances								
		$1:10^2$	$1:2\times10^2$	$1:4\times10^2$	$1:5\times10^2$	$1:8\times10^2$	$1:10^3$	$1:1.6\times10^3$	$1:5\times10^3$	$1:10^4$
1	A	−			±		+		++	+++
	B	+			++		++		+++	+++
2	A	−			−		+		++	+++
	B	+			++		++		++	+++
3	A	−			−		+		++	+++
	B	++			++		++		+++	+++
4	A	−	−	−		±		+		
	B	+	+	+		+		+		
5	A	−	−	−		+		+		
	B	+	+	+		++		++		
6	A	−			−		+		++	+++
	B	++			++		++		+++	+++
7	A	−	−	−		−		+		
	B	+	+	+		+		+		
8	A	−	−	−		+		+		
	B	+	+	++		++		++		
9	A	−	−	+		+		++		
	B	+	+	+		++		++		
10	A	−	−	−		+		+		
	B	+	+	+		++		++		
11	A	−	−	−		+		+		
	B	+	+	+		+		++		
12	A	−	−	−		−		+		
	B	+	+	+		+		++		
13	A	−	+	+		++		++		
	B	+	+	+		++		++		
14	A	−	−	−		−		+		
	B	+	+	+		+		+		

* Signifies the same as ‡ in Table I.

centrifuged supernatant was distilled to dryness (**III**). The alcohol portions used for the preservation were treated likewise, and the still residues thereby (**I**'s) and the substances recovered from the hot alcoholic extracts were all washed with acetone and dried. **III**, washed with acetone, of all the pancreases were found to be specifically anti-isoagglutinative to **A** erythrocytes. **I**'s, washed with acetone, caused noticeable hemolysis so that definite judgement of the agglutination was allowed only in some of those tested, which indicated that the Group **A** lipoid had entered partially into the cold alcohol. The other fractions including the acetone-soluble and the tissue residues were inactive. A great part of the details of the experiment is embodied in Tables I, II and III.

Isolation of the Group Lipoid

1st stage. The balance of Fr-**III**'s, washed with acetone, of the pig pancreases above were combined (10.5 g. altogether) and exhausted with three 150 cc. portions of ether (cold) and the solutions were distilled in vacuo and dried in a desiccator—Fr. **Sol**$_{et}$ (a yellowish butterlike substance, 0.15 g.). It showed no inhibition of the isoagglutination of **A** and **B** erythrocytes at a dilution of 1:200 (Complete hemolysis occurred at 1:100 dilution of the substance). The insoluble part—Fr. **Ins**$_{et}$ (a yellowish, non-sticky powder, 9.9 g.) was Group **A** active (inhibitory to isoagglutination of **A** erythrocytes at 1:500), but not reactive with β-agglutinin at 1:100. This was further divided, employing three 150 cc. portions of chloroform. The chloroform-soluble (Fr. **Sol**$_{chl}$), recovered from the solutions similar to above, amounted to 0.5 g. (a brownish, hard clump). When it was added, as dissolved in physiological saline, to the

Fig. 1. An electrophoretic pattern of a 0.86% solution of Fr. Ins$_{chl}$ in phosphate buffer, pH 7.8, I=0.29; 5°C; current 10 ma. Exposure 60 minutes after starting current. Ascending limb to the right (Boundaries were shifted before photographing).

TABLE IV

Occurrence of Isohemoagglutination, when Fractions at the First Stage of Preparation had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively.
−: no agglutination, +: slight aggl., ++: marked aggl., ###: complete aggl., H: hemolysis.

Fraction	Group of red cells	Dilution of substances									
		1: 10^2	1: 2×10^2	1: 4×10^2	1: 5×10^2	1: 8×10^2	1: 10^3	1: 1.6×10^3	1: 5×10^3	1: 10^4	1: 5×10^4
Sol$_{et}$*	A	H	++	###		###		###			
	B	H	###	###		###		###			
Ins$_{et}$*	A	H			−		+		+	+	+
	B	+			+		++		###	###	###
Sol$_{chl}$*	A	H	±	+		+		++			
	B	+	+	++		++		###			
Ins$_{chl}$*	A	H			−		+		+	+	+
	B	+			++		++		++	++	++

* See the text.

normal antisera, **A** erythrocytes agglutinated only faintly at a dilution of 1:200 of it in the saline (hemolysis at 1:100) but **B** erythrocytes agglutinated markedly at its 1:100 dilution. The residue (Fr. $\mathbf{Ins_{chl}}$)—a yellowish, hygroscopic, fine powder—had the same Group **A** potency as the ether-insoluble, and gave positive Molisch and biuret reaction, and electrophoresis of a 0.86% solution (somewhat turbid) in a buffer of pH 7.8 demonstrated two components (Fig. 1). This was mostly soluble in cold and hot methanol, cold neutral- and faintly-alkalinized-water, and hot acidulated water. Completely soluble in hot neutral- and faintly-alkalinized-water. Insoluble in ether, petrol ether and chloroform.

(Serological potencies of the fractions above are illustrated in detail in Table IV.)

2nd stage. It was kneaded with a little water and water added up to 300 cc., followed by vigorous agitation. The centrifugate was further exhausted with two 300 cc. portions of water. The clear yellowish supernatant were united (100 cc. altogether) and added to with 90 cc. of 5% lead acetate, and the centrifugate here washed with changes of a dilute solution of the salt with centrifugation. The supernatant were bubbled with H_2S. The black precipitate was separated and washed with hot water and the mother fluid and washings were distilled together to dryness and washed with acetone. 7.5 g. of a yellowish hygroscopic, fine powder ($\mathbf{Non\text{-}ppt_{Pb}}$) were given which was group-inactive. The lead precipitate in 900 cc. of water was also treated with H_2S and the suspension was acidified to pH 1.0 with HCl. Centrifuged. The transparent yellowish supernatant was neutralized with NaOH, distilled and washed with acetone. And 0.6 g. of a brownish, very hygroscopic powder was given—$\mathbf{Ppt_{Pb}\text{-}Sol_{HCl}}$. The centrifugate was washed with three 50 cc. portions of a very dilute HCl (pH 1.0) and then extracted repeatedly with methanol. In the second and following times, the extractant was added to with HCl to pH 6.0 to obtain a clear centrifuged supernatant. The solutions were concentrated by vacuum distillation, neutralizing halfway and rejecting some precipitate thereby, to dryness. The remainder was washed with acetone. 670 mg. of a non-sticky powder with light yellow-brown shade were given—$\mathbf{Ppt_{Pb}\text{-}Ins_{HCl}}$. This product inhibited isoagglutination of **A** erythrocytes (complete inhibition at a dilution of 1:1000), but not that of **B** erythrocytes. Molisch positive, biuret negative. The electrophoresis revealed it was still heterogeneous. Here a 0.86% solution in an alkaline buffer was applied and two boundaries were demonstrated (Fig. 2). It was soluble partially in cold methanol, ethanol, benzene, tetralin and water, but mostly in hot methanol, ethanol and water and completely in hot tetralin and alkaline water. Insoluble was it in ether, petrol ether and chloroform.

TABLE V

Occurrence of Isohemoagglutination, when Fractions at the Second Stage of Preparation had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively.
−: no agglutination, +: slight aggl., ++: marked aggl., ##: complete aggl., H: hemolysis.

Fraction	Group of red cells	Dilution of substances										
		1: 10^2	1: 2×10^2	1: 4×10^2	1: 5×10^2	1: 8×10^2	1: 10^3	1: 1.6×10^3	1: 5×10^3	1: 10^4	1: 2×10^4	1: 4×10^4
Non-ppt$_{Pb}$*	A	##	##	##		##		##				
	B	##	##	##		##		##				
Ppt$_{Pb}$-Sol$_{HCl}$*	A	++	##	##		##		##				
	B	##	##	##		##		##				
Ppt$_{Pb}$-Ins$_{HCl}$*	A	−			−		−		+	+	+	++
	B	H			+		++		++	++	##	##

* See the text.

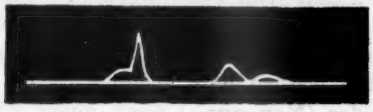

Fig. 2. An electrophoretic pattern of a 0.86% solution of Ppt$_{Pb}$-Ins$_{HCl}$ in phosphate buffer, pH 7.8, I=0.29 (The substance was dissolved in the buffer by warming at 30°C for 2 minutes.); 5°C; 10 ma. Exposure 40 minutes after starting current. Ascending limb to the left.

3rd stage. 0.3 g. of it was subjected to the procedure of counter current distribution, employing 10 funnels. Solvent pair, an equilibrated mixture of methanol, benzene, toluene and water (4:4:1:1). The substance was at first dissolved in 150 cc. of the lower phase and distributed between that solvent and an equal volume of the upper phase. After the last transfer the content of all the funnels were distilled separately and the residuals washed with acetone and dried.

	Funnel No.									
	0	1	2	3	4	5	6	7	8	9
Weight of the products (mg.)	6	6	11	15	15	17	15	15	20	130

Their anti-isohemoagglutinative potencies are illustrated in Table VI, which shows the fraction from Funnel 9 only was definitely group (**A**)-active.

TABLE VI

Occurrence of Isohemoagglutination, when Fractions in the Third Stage of Preparation had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively.

−: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., +++: complete aggl., H: hemolysis.

Fraction*	Group of red cells	Dilution of substances											
		1:10^3	1:2×10^3	1:4×10^3	1:5×10^3	1:8×10^3	1:10^3	1:1.6×10^3	1:2×10^3	1:3.2×10^3	1:4×10^3	1:6.4×10^3	1:8×10^3	1:1.2×10^4
0	A		+	+		+		++		++				
	B		+	+		+		+		+				
1	A		+	+		+		+		+				
	B		+	+		+		+		+				
2	A		+	+		+		+		+				
	B		++	++		++		+++		+++				
3	A		+	+		+		+		+				
	B		++	++		+++		+++		+++				
4	A		+	+		+		+		+				
	B		+	+		++		+++		+++				
5	A		+	+		+		+		+				
	B		+	+		+		++		++				
6	A		+	+		+		+		+				
	B		+	+		++		+++		+++				
7	A		H	H		H		+		+				
	B		H	+		+		+		+				
8	A				H		H		+		+		+	
	B				H		+		+		+		+	
9	A				H		H		−		±		+	
	B				+		+		+		+		+	
9-Ins†	A	H	H	H		H		H		H				
	B	H	H	H		H		H		H				
9-Sol†	A	H	H	H		H		−		±		+		+
	B	H	H	+		+		+		++		++		++

* Figures express funnel numbers. † See the text.

In a similar run with the balance of the product in the foregoing stage (320 mg.), 140 mg. of a substance, washed with acetone, were recovered from Funnel 9.

The substances yielded in the both runs (265 mg. altogether) were shaken with 30 cc. of water on an apparatus for 6 hours and centrifuged. On the centrifugate was repeated the operation many times (300 cc. of water used in total). The insoluble part (a powder tinged yellowish) —**9-Ins**—amounted to 15 mg., and in the examination of the group

activity it caused complete hemolysis even at the dilution of 1:3,200 (Table VI). The solutions were distilled in vacuo and washed with acetone—240 mg. of a yellowish non-sticky powder (**9-Sol**) were given. This product was specifically Group **A** active. It also exhibited hemolytic action, though not strong, probably due to a trace of the water-insoluble part contaminating (Table VI). Electrophoretically it was homogeneous as is shown in Fig. 3.

Fig. 3. An electrophoretic pattern of a 0.67% solution of 9-Sol in phosphate buffer, pH 7.8, I=0.29; 11°C; 15 ma. Exposure 60 minutes after starting current. Mobility 2.03 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. Ascending limb to the left.

Since, however, it contained an abundant amount of ash (64.4%, Pregl's method without use of H_2SO_4), 130 mg. of it were electrodialyzed as dissolved in 7 cc. of water (Pauli apparatus) until no shift of pH occurred in the outer fluids (2 days requisite). The substance precipitated thereby in part, but because of difficulty in separating the precipitate, the solution containing it was distilled as such in vacuo and dehydrated with acetone. A yellowish powder weighing 30 mg. was provided. Biuret, Goldschmiedt (for hexuronic acid), Neuberg & Saneyoshi (for hexuronic acid) and Rosenthaler (for methylpentose) negative; Molisch and Elson & Morgan positive. Composition, group-activity and solubilities are shown in Tables VII, VIII and IX.

TABLE VII

Composition of the Final Preparation of Pig Pancreas Group Lipoid

Analysis	In per cent	In equiv. per equiv. wt.
N	4.2	2.9
P	3.2	1.0
Ferricyanide reduction of the hydrolystate* as glucose	9.2	
Ash†	3.5	

* The substance was hydrolyzed by 4 hours' heating with 1 *N* H_2SO_4, as sealed in an ampoule. The hydrolysate contained none precipitable by phosphotungstic acid so that it was immediately neutralized for measurement of the reduction. † Pregl method without H_2SO_4.

Table VIII

Occurrence of Isohemoagglutination, when the Final Preparation had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80, and were diluted 20 and 5 times respectively.
−: no agglutination, +: slight aggl., ++: marked aggl., ###: complete aggl., H: hemolysis.

Group of red cells	Dilution of the substance					
	1: 5×10^2	1: 10^3	1: 2×10^3	1: 4×10^3	1: 8×10^3	1: 1.6×10^4
A	H	H	−	+	+	+
B	+	+	+	+	++	###

Table IX

Solubilities of the Final Preparation

−: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol., ###: completely sol., cold=20°C, hot=50–60°C.

Solvents	Water						·Methanol		Ethanol		Ether		Petrol ether		Benzene		Glacial acetic acid		Tetralin		Chloroform	
	acid*			plain		alkaline†																
	cold	hot		cold	hot		cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubilities	−~ ±	−~ ±		+	+~ ++	##‡	###	±	±	±	±	−	−	−	−	±	±	−	+	+	++	−	−

* pH<1.2, † pH>9.6, ‡ A slightly opalescent solution formed.

Compared with the corresponding substance of human pancreas, the following remarks are given as to the present group lipoid:—1) it is much less group-active; 2) the potency is not diminished by the third stage-operation; 3) the equivalent ratio of N and P of it (2.9:1.0) does not agree with that of the one in human pancreas (1.9:1.0); 4) it contains less carbohydrate and more N and P than the human substance.

Summary

14 pig pancreases contained all a Group **A** lipoid. The tissue residues after extraction with hot alcohol was group-inactive.

The group lipoid was prepared in an electrophoretically homogeneous state. It analyzed: N 4.2%, P 3.2%, ferricyanide reduction of the hydrolysate as glucose 9.2%, ash 3.5%; and inhibited specifically the isoagglutination of **A** erythrocytes at a dilution of $1:2\times10^3$. It dissolved

in alkaline water entirely and in hot tetralin mostly, but did not dissolve in ether, petrol ether and chloroform.

Through the Grant Committee of the Science Council was given a grant from the Education Department, which aided this work. H. Masamune.

References

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Biochemical Studies on Carbohydrates

CLIX. High-molecular Components of Non-lipid Nature of Human Pancreas

First Paper: Carbohydrates

By

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From human pancreases from persons belonging to Group **A**, three hexosamine-containing carbohydrates (preparations) were separated. Glucidamins appeared absent or to amount very small, if present. The nucleic acids, whose crude products have been obtained, will be dealt with in a forthcoming article.

Experimental

Fractionation Procedure

Tokura[1] dehydrated the pancreases from 20 Group **A** persons and boiled them with denatured alcohol for extraction of lipids. The tissue residues thereby, weighing 155 g., were applied here as a material and shaken with 1.5 *l.* of 10% NaCl for 3 days, employing toluene as a preservative. The centrifugate was furtheron treated likewise with one 1.5 *l.* and four 1 *l.* portions of the same saline solution. The sixth extract gave no Bial's orcinol reaction.

The residual in this place was exhausted with plain water repeatedly, and 0.63 g. of a substance was obtained, which was biuret-positive and slightly Molisch-positive but Bial-negative and contained no phosphorus. It was subdivided by saturation of its solution with $(NH_4)_2SO_4$. From the precipitate and mother fluid were respectively recovered 0.29 g. of a substance (a gray powder) that was devoid of phosphorus and Bial-, Dische- and Neuberg & Saneyoshi-negative, faintly Molisch-positive and strongly biuret-positive, and 35 mg. of a substance (a white powder) that was weakly biuret-positive, strongly Molisch-positive and free from phosphorus. The latter fraction was mostly insoluble in glacial acetic acid and appeared to involve a carbohydrate or carbohydrates in chief, but because of too small an amount, it was not further studied.

The supernatant fluids (turbid) after centrifugation of the saline extracts were fractioned separately at first in a manner, as will be described.

The fluid obtained at first (1.25 *l.*) was yellowish-brownish colored, opaque and weakly acid (pH 6.0). On addition to it of 10% HCl to a pH of 1.5, a voluminous flocculent precipitate came out, which was separated and washed with a dilute HCl of the same pH.

It was agitated in 45 cc. of 10% NaCl and 10% NaOH added to neutral, and to the somewhat turbid supernatant after centrifugation were added 5 volumes of 99% denatured alcohol. The precipitate was subjected twice more to the extraction and precipitation, and the final deposit, washed with alcohol, was dried. 0.72 g. of a yellowish brownish powder was given —**Cr. NA I_1.** By fractionation with barium acetate, baryta, a conc. NaCl solution and alcohol was shown that it consisted mainly of nucleic acids.

The mother fluid and washings were dialyzed against running water for 3 days, whereby the pH was elevated to 4.8 (Some deposit appeared, but it was not eliminated), and distilled in vacuo to about 30 cc. The condensate was neutralized and centrifuged. The supernatant was not perfectly clear. On addition of HCl to pH 1.5, a voluminous precipitate came out which was separated and set aside (**Cr. NA II_1,** 1.02 g.). The clear mother fluid obtained was adjusted to pH 6.0 (with NaOH) and distilled in vacuo to about 5 cc. and 50 cc. of 99% denatured alcohol and a little anhydrous sodium acetate added. The occurring precipitate was washed well with alcohol and dried in a desiccator—a yellowish, non-sticky powder, 4.5 g.—**Cr. Ch. I_1.**

From the second to sixth saline extracts were given decreasing amounts of the corresponding products (in total 2.2 g.)—**Cr. Chs. I_2–I_6.**

Cr. Chs. I_1-I_6 were united—**Cr. Ch. I.** Biuret, Molisch, Bial's orcinol and Dische's diphenylamine test resulted all in positive. An appreciable quantity of phosphorus was detected.

5.56 g. of Cr. Ch. I were taken up in 120 cc. of water. The turbid solution (pH 5.8) was alkalinized (to pH 9.2) and the flocculent precipitate, composed mainly of inorganic salts, was discarded after centrifugation. The transparent supernatant was acidulated (to pH 4.8) with HCl and 15 cc. of 20% $CuCl_2$ added (The pH of the solution lowered to 3.8). The abundant precipitate here was separated.

This was washed with 2% HCl and suspended in 5 cc. of water, followed by addition of 3 drops of 10% HCl and 7 volumes of abs. denatured alcohol, containing 0.5 cc. of 35% HCl in 100 cc. ("0.05 *N* alcoholic HCl"), and centrifugation. The centrifugate was treated in a similar manner twice more, well washed with alcohol and dried. A slightly blue-colored powder weighing 0.67 g. was given—**Cr. NA III.** It contained much phosphorus and gave strongly positive Bial (for pentose) and Dische (for desoxypentose) reaction. It showed positive biuret and Molisch reaction besides. Neuberg-Saneyoshi, almost negative.

The green supernatant was dialyzed against running water for 3 days, whereby it changed turbid (pH 5.2), and distilled in vacuo to about 30 cc. (pH 4.6). A voluminous precipitate appeared. This was centri-

fuged and treated as the precipitate by $CuCl_2$ above. 0.67 g. of a substance was given—**Cr. NA IV.** It showed not only positive reactions of nucleic acids but also comparatively strong Neuberg & Saneyoshi reaction, and hence it was used as one of the materials to prepare the hexuronic acid-containing carbohydrate (See below, p. 35). The supernatant fluid was condensed furtheron to 15 cc., acidified with 5 drops of 10% HCl and after addition of a little NaCl, precipitated with 10 volumes of the 0.05 *N* alcoholic HCl. Reprecipitated twice, washed with alcohol and dried. A powder tinged yellow-brown was given. 1.39 g. Phosphorus was present, though very little.

Fractionation with $(NH_4)_2SO_4$. 1.37 g. of the product immediately above were taken up in 15 cc. of water. To the slightly turbid solution of pH 4.8 was added NaOH to pH 5.6. Following saturation with $(NH_4)_2SO_4$ caused marked precipitation. The saturated $(NH_4)_2SO_4$ was further added up to 45 cc. and the mixture stood overnight. And the precipitate was collected on a funnel and washed with the saturated $(NH_4)_2SO_4$. The filtrate and washing (70 cc. in total) were diluted with water and dialyzed for 5 days. Since the sulfate ions were found still remaining, the solution was distilled to about 60 cc. in vacuo to dialyze again. In 2 days the sulfate ions were completely removed. The solution was then condensed to 10 cc. (pH 6.0), 10 volumes of alcohol and a little anhydrous sodium acetate added and the deposit washed with alcohol and dried. 0.5 g. of a white powder was provided—**Cr. Ch. I′.** Ash 3.24%. It was not free from biuret-giving substances. Molisch strongly positive. Neuberg-Saneyoshi reaction very faint. Phosphorus was not detected. On the other hand, the $(NH_4)_2SO_4$-precipitate was dissolved in 60 cc. of water. A yellow-brown, somewhat turbid solution (pH 5.6) formed, which was dialyzed for 5 days, distilled in vacuo to 10 cc. and precipitated with 10 volumes of alcohol, followed by washing with alcohol and drying. The white powder (0.47 g.) given—**$Ppt_{(NH_4)_2SO_4}$ I**—was strongly biuret-positive and, besides, gave the positive Molisch and Neuberg-Saneyoshi reaction. Phosphorus was detected, though not distinct. From this fraction also, the uronic acid-containing polysaccharide was separated as below (p. 35).

Subdivision of Cr. Ch. I′ with glacial acetic acid. 0.42 g. of Cr. Ch. I′ was kneaded with 5 drops of water to a paste and 15 cc. of glacial acetic acid (b.p. 118–118.5°C) added in small portions. The flocculent precipitate was separated, washed with two 5 cc. portions of the conc. acetic acid at first and afterwards with 5 changes of ether and dried—**Cr. Ch. I′A** (A white powder, 198 mg.). The mother fluid (yellowish) and glacial acetic acid-washings were united and precipitated with 3 volumes of ether, and after thorough washing with ether, dried —**Cr. Ch. I′B** (a

non-colored powder with waxy appearance, 192 mg.). Cr. Ch. I'A: biuret faintly positive, Molisch strongly positive, Neuberg & Saneyoshi and Bial weakly positive. Cr. Ch. I'B: biuret positive, Molisch strongly positive, Neuberg & Saneyoshi negative, Bial feeble.

Purification of Cr. Ch. I'A. 190 mg. of the crude substance were again subjected to kneading with a little water (3 drops) and following precipitation with glacial acetic acid (10 cc.) repeatedly until the mother fluid showed no turbidity on addition of ether. (The precipitates from the mother fluids by ether were small in amount.) Seven repetitions of the operation were requisite. The product, washed with ether and dried, (a white hygroscopic powder) amounted to 157 mg. Of it 150 mg. were taken up in 10 cc. of water and a small insoluble part rejected. The translucent solution (pH 4.0) was adjusted to pH 5.6 with NaOH and 5 drops of the saturated lead acetate (pH 5.6) were added, whereby an abundant flocculent precipitate came out. Stood overnight. The precipitate was separated by centrifugation and washed with a dilute lead acetate solution. The mother fluid and washings were united (18 cc. in total), bubbled with H_2S and filtered, then, the water-clear filtrate was concentrated to about 2 cc. (pH 6.0), to which 10 volumes of alcohol and a little alcohol saturated with anhydrous sodium acetate were added. The deposit here was washed with changes of alcohol (together with anhydrous sodium acetate when necessary), followed by drying in a desiccator. A white powder (102 mg.) was given—**Ch. IA.** Biuret negative; Molisch intensively positive; the positive Osaki & Turumi reactions, direct and indirect, given; Neuberg & Saneyoshi negative; Bial almost negative; phosphorus absent.

Purification of Cr. Ch. I'B. 180 mg. of the crude product were kneaded with 4 drops of water and 10 cc. of glacial acetic acid were added. Some deposit was discarded. The mother fluid was precipitated with 3 volumes of ether, washed well with ether and dried. The substance given was treated likewise 5 times more, when none glacial acetic acid-precipitable remained. The product at this stage amounted to 106 mg. This was further shaken with 5 cc. of the glacial acetic acid in a stoppered flask for 3 hours. Centrifuged. The centrifugate was shaken with 3 cc. of the glacial acetic acid and again centrifuged. The supernatant fluids were united and precipitated, washed and dried as above. The substance given (78 mg.) was then taken up in 3 cc. of water and the insoluble part was again treated with 2 cc. of water. The solutions were precipitated with 5 volumes of alcohol, washed with alcohol and dried. A white powder with waxy appearance (72 mg.) was thus given—**Ch. IB.** Molisch strongly positive, the direct and indirect Osaki & Turumi reactions weakly positive, biuret almost negative, Neuberg & Saneyoshi

and Bial perfectly negative, phosphorus absent.

Rough separation of the hexuronic acid-containing carbohydrate from Cr. NA IV. 0.63 g. of this crude nucleic acid-fraction was exhausted with one 10 cc. and three 5 cc. portions of water. The solutions (25 cc., pH 6.8, a little turbid) were acidulated (to pH 4.8 with 10% HCl) and enough 20% $CuCl_2$ added to remove all the precipitable (8 drops of the $CuCl_2$ solution required). Stood overnight. The precipitate was centrifuged and washed with a dilute $CuCl_2$ solution with subsequent centrifugation. The supernatant fluids (transparent and green, 35 cc. in total) were added to with 5 drops of 10% HCl, and precipitated with 7 volumes of the 0.05 *N* alcoholic HCl. The precipitate here was further subjected to a similar operation 3 times and after washing with alcohol 4 times, dried, whereby 128 mg. of a substance were yielded. Next, 122 mg. of the substance were kneaded with 2 drops of water to a paste and 10 cc. of glacial acetic acid added in small portions. And the precipitate was washed with the conc. acetic acid twice and with ether 5 times and dried. Then it was dissolved in 0.3 cc. water, precipitated with 3.4 cc. of the glacial acetic acid and after washing with two changes of 90% acetic acid and five changes of ether, dried. Another reprecipitation was carried out employing 0.5 cc. water and 2 cc. of glacial acetic acid (99%), and the deposit washed with two changes of 80% acetic acid. After thorough washing with ether, 75 mg. of a white substance, which had some fibrous appearance, were obtained—**Cr. Ch. II_a.**

Rough separation of the hexuronic acid-containing carbohydrate from $Ppt_{(NH_4)_2SO_4}$ I. 0.45 g. of the material was dissolved in 10 cc. of water. To the yellowish brownish, slightly turbid solution of pH 7.0 was added 10% HCl to pH 1.0. The occurring precipitate was centrifuged and washed with a dilute HCl of pH 1.0.

It amounted to 83 mg. Biuret positive; Neuberg & Saneyoshi reaction faint. The Molisch reaction test also resulted in positive, though weak, which was probably due to the pentose in the nucleic acid because the substance was Bial-positive and contained phosphorus.

The clear supernatant and washing of the centrifugate (18 cc. altogether) were adjusted to pH 5.6 with NaOH and mixed with an equal volume of the saturated $(NH_4)_2SO_4$. A pretty voluminous precipitate separated. This was washed with the half-saturated $(NH_4)_2SO_4$.

The substance insoluble in the half-saturated $(NH_4)_2SO_4$ (recovered by dialysis, condensation and precipitation with alcohol) amounted only to 65 mg. Biuret positive, Molisch and Neuberg & Saneyoshi weak, phosphorus absent.

The mother fluid (opalescent) and washing (23 cc. altogether) were then saturated with $(NH_4)_2SO_4$ and stood overnight. The precipitate hereby was separated, washed twice with the saturated $(NH_4)_2SO_4$ and

after dissolving in 35 cc. of water, dialyzed for 5 days. The solution (somewhat turbid, 70 cc., pH 6.2) was distilled under diminished pressure to about 5 cc., precipitated with 10 volumes of alcohol, washed with alcohol and dried—**Ppt$_{(NH_4)_2SO_4}$ I′** (a yellowish-brownish powder, 178 mg.). Biuret, Molisch, Neuberg & Saneyoshi all positive; phosphorus absent.

90 mg. of a substance were recovered from the $(NH_4)_2SO_4$-saturated mother fluid by dialysis, condensation and addition of alcohol. This showed weak biuret reaction contrary to strong Molisch, so that it was thought to be a fraction resembling Cr. Ch. I′ above.

150 mg. of Ppt$_{(NH_4)_2SO_4}$ I′ were then treated with a little water and glacial acetic acid as directly above, decreasing gradually the concentration of acetic acid at which the fractionation was effected. 60 mg. of a white fibrous product were obtained. This was taken up in 5 cc. of water, 2 *N* NaOH added to pH 6.2 and a small insoluble part centrifuged, washed and rejected. The clear supernatant and washing (in total 8 cc.) were diluted to 15 cc. and dialyzed for 2 days to make perfectly sulfate-free. After distillation to 3 cc., it was precipitated with alcohol (5 volumes), washed with alcohol and dried. The amount of substance was reduced to 38 mg.—**Cr. Ch. II$_b$.**

Most of the Molisch-giving substance or substances was eliminated by the glacial acetic acid-treatment. The solutions thereby were combined and precipitated with ether. It is not excluded that a glucidamin or glucidamins were present in this fraction, but because of too small a crop and Neuberg & Saneyoshi-positiveness, further study of the fraction was not tried.

Purification of the hexuronic acid-containing carbohydrate. Cr. Ch. II$_a$ and Cr. Ch. II$_b$ behaved similar in qualitative tests: biuret not strong (II$_b$ showed only weak reaction), Molisch faint, Goldschmiedt, Neuberg & Saneyoshi and Bial pretty strong, phosphorus and inorganic sulfate absent, hydrolysable sulfur present. Therefore, they were put together to purify by the Sevag process:—100 mg. of the united substance (II$_a$, 68 mg.+II$_b$, 32 mg.) were taken up in 5 cc. of water and 10% NaOH added to pH 5.8. The centrifuged supernatant and washing of the centrifugate (10 cc. in all, somewhat turbid) were shaken with 2.6 cc. of a mixture of chloroform and toluene (3:1 by volume) for 30 minutes. The centrifuged gel phase was washed with one 5 cc. and one 3 cc. portion of water. The washings were added to the watery phase and the solution mixture (20 cc. in all) were condensed to half the volume for a similar treatment with the organic mixture. The operations were repeated 6 times more. And a water-clear solution given was concentrated to about 3 cc. (still clear here), precipitated with 5 volumes of alcohol and a little anhydrous sodium acetate, and well washed with alcohol. The more or less fibrous product weighed 62 mg. when dried—**Ch. II.** Biuret negative, Molisch

almost negative, phosphorus not detected, Goldschmiedt and Neuberg & Saneyoshi positive.

Properties of the Carbohydrates

Solubilities

Ch. IA. Soluble in water at all pH's. It also dissolved in 10% trichloroacetic acid and the saturated $(NH_4)_2SO_4$. Not precipitated by cupric chloride, lead acetate, barium acetate and baryta. Insoluble in glacial acetic acid.

Ch. IB. Soluble in water (at all pH's) and glacial acetic acid. In the sat. $(NH_4)_2SO_4$, a minute part did not go into solution. It contained none precipitable by cupric chloride, lead acetate, barium acetate and baryta.

Ch. II. Soluble in water at all pH's. Contained none precipitable by cupric chloride. Entirely insoluble in 80% or more concentrated acetic acid.

Quantitative composition

The analytical figures are embodied in Table I.

TABLE I

Composition of the Pancreas Carbohydrates (preparations)

Analysis	In per cent			In equiv. per equiv. wt.		
	Ch. IA	Ch. IB	Ch. II	Ch. IA	Ch. IB	Ch. II
N	4.0	5.2	2.9	1.6	2.7	1.0
Hexosamine	32.9	24.3		1.0	1.0	
Non-amino sugars						
as galactose	34.6*	41.3*, 37.8†		1.0	1.7, 1.5	
as mannose	37.9*	43.7*, 41.1†		1.1	1.8, 1.7	
as glucuronic acid			38.4*			1.0
as galacturonic acid			40.1*			1.0
Hydrolysable sulfur			1.7‡			0.3
Ash§	8.7	1.0	11.7			

* The orcinol method. † The indole method. ‡ Estimated gravimetrically as $BaSO_4$. § Pregl method without use of H_2SO_4.

Ch. II seemed to involve glucosamine (See below), glucuronic acid (See below) and sulfuric acid in the molecular proportions of 1:1:0.3. The low value of hydrolysable sulfur is probably ascribed either to the analytical error (Only 7.3 mg. of the substance were taken for the gravimetric estimation) or to being a mixture of an itin and an itin-sulfuric acid of the preparation. Shortage of material did not allow an accurate study.

Optical rotation (in water)

Ch. IA: $[\alpha]_D^{16.5}=\frac{-0.199\times 1}{0.0126\times 1}=-15.8°$.

Ch. II: $[\alpha]_D^{12.8}=\frac{-0.365\times 1}{0.0104\times 1}=-35.1°$.

Blood group activity

Ch. IA specifically inhibited the isoagglutination of **A** erythrocytes, but Ch. IB did not (Table II). The method of the assay was the one usual to us.

TABLE II

Occurrence of Isohemoagglutination when either Ch. IA or Ch. IB had been added to the Normal Anti-sera

The **B** and **A** serum had agglutinin titres of 320 and 80 and were diluted 20 and 5 times respectively. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., ###: complete aggl.

Carbohydrate	Group of red cells	Dilution of substances											
		1: 5×10^2	1: 10^3	1: 2×10^3	1: 3×10^3	1: 4×10^3	1: 6×10^3	1: 8×10^3	1: 1.2×10^4	1: 1.6×10^4	1: 2.4×10^4	1: 3.2×10^4	1: 4.8×10^4
IA	A	−	−	−		±	+	+	+	+	+	+	+
	B	+	++	++		###		###					
IB	A	++	++	###	###	###	###	###	###	###	###	###	###
	B	###	###	###	###								

Paper partition chromatography

The spots in the chromatograms indicated the presence of glucosamine, chondrosamine, galactose, mannose and L-fucose in either of Chs. IA and IB (Figs. 1, 2 and 3) and glucosamine and glucuronic acid in Ch. II. Irrigation was effected according to Masamune and Maki.[2] Developer, aniline-phthalic acid of Partridge.

Ch. I. 35 mg. of this carbohydrate (specimen) were heated with 3.5 cc. of 1 *N* H_2SO_4 in a boiling water-bath under reflux. Duration of hydrolysis 5½ hours. The hydrolysate was diluted twofold and after addition of baryta (powder and the saturated solution) to pH 4.6 (Some sulfuric acid was left unprecipitated), centrifuged, and the centrifugate was washed with hot water. The mother fluid and washings (yellowish-brownish) were distilled in vacuo to 0.5 cc. 0.02 cc. portions of the condensate were put on paper strips for irrigation. Solvents: 1) *n*-butylacetate-acetic acid-butanol-methanol-water (3:2:2:1:1), 2) *n*-butylacetate-

Fig. 1

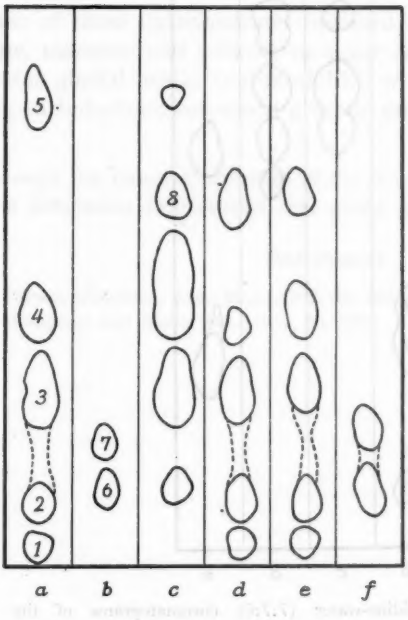

Fig. 2

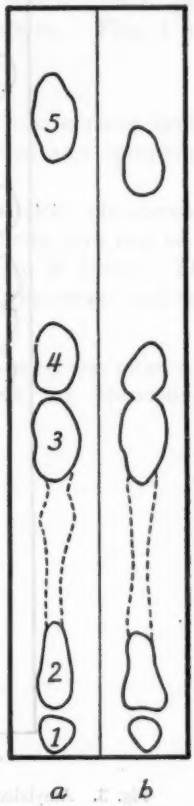

Fig. 1. *n*-Butylacetate-acetic acid-butanol-methanol-water (3:2:2:1:1) chromatograms of the pancreas carbohydrates. Solvent fronts run 45 cm. Temperature of test 25°C.

a, b and c (a hydrolysate of traganth): standards, d: Ch. IA, e: Ch. IB, f: Ch. II. 1: chondrosamine sulfate, 2: glucosamine sulfate, 3: galactose, 4: mannose, 5: rhamnose, 6: galacturonic acid, 7: glucuronic acid, 8: L-fucose.

Fig. 2. *n*-Butylacetate-acetic acid-water (3:2:1) chromatogram of one of the pancreas carbohydrates (Ch. IA). Solvent front run 45 cm. Temperature of test 25°C.

a: standard, b: Ch. IA, 1–5 signify the same as in Fig. 1.

acetic acid-water (3:2:1), 3) amylalcohol-pyridine-water (7:7:6). (Figs. 1, 2 and 3)

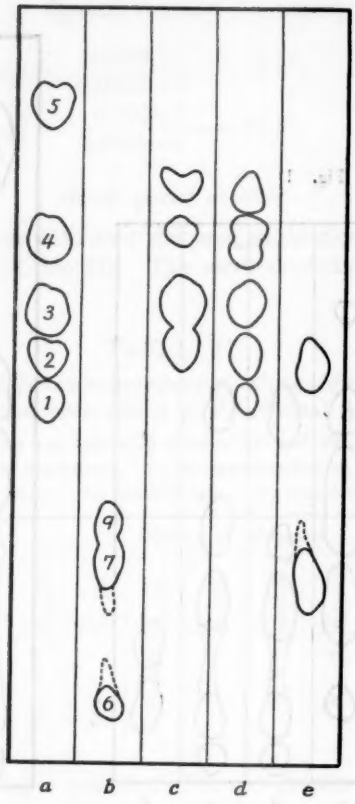

Fig. 3. Amylalcohol-pyridine-water (7:7:6) chromatograms of the pancreas carbohydrates. Solvent fronts run 40 cm. Temperature of test 30°C.

a and b: standards, c: Ch IA, d: Ch. IB, e: Ch. II. 1–7 signify the same as in Fig. 1. 9: mannuronic acid.

Ch. IB. 29 mg. of the substance were hydrolyzed with 3 cc. of 1 *N* H_2SO_4 in a similar manner as Ch. IA. After removal of most of H_2SO_4 by addition of baryta to a pH of 4.8, the hydrolysate was condensed to 0.4 cc. and 0.02 cc. portions were applied for irrigation. Solvents, 1) and 3) above. (Figs. 1 and 3)

Ch. II. 36 mg. of the substance were dissolved in 0.4 cc. of a mixture of conc. H_2SO_4 (sp. gr. 1.84) and water (5:2 by volume) and stood as stoppered for 96 hours in an ice chest. The hydrolysate was diluted five times with water, and baryta added to pH 2.0. The centrifugate was

washed with water with following centrifugation and the supernatant fluids united and distilled in vacuo to 0.3 cc., of which 0.04 cc. portions were put on paper strips. Solvents, 1) and 3) above. (Figs. 1 and 3)

Summary

Extracts with 10% NaCl from pancreases (from persons belonging to Group **A**) were fractioned and three carbohydrates (preparations) were obtained.

Two of those carbohydrates involved glucosamine, chondrosamine, galactose, mannose and L-fucose as sugar components, and one of them, which was glacial acetic acid-insoluble, was Group **A** active. The remaining carbohydrate contained glucosamine and glucuronic and sulfuric acid.

Through the Grant Committee of the Science Council was given a grant from the Education Department that aided this work. H. Masamune.

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Comparative Observation of the Action Potential of Autonomic Nerve with E.E.G.

By

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Correlation between the brain and the autonomic nerves have been studied by many authors from anatomical as well as physiological standpoints, but still many there are left to be clarified.

A great number of articles of E.E.G. have been published after the discovery of E.E.G. by Berger, and contributed much both to the brain physiology and clinical medicine. On the other hand, enormous advance in our understanding of the action potentials of peripheral nerves was established by many investigators following the brilliant works by Erlanger & Gasser. Standing on such a ground of recent advance in neurophysiology, correlations of brain and peripheral nerves were studied by some authors, that is, studies of brain and cord by Davis etc.,[1),2),3),4)] those of brain and peripheral nerves by Gibbs,[5)] Gellhorn,[6)] and those concerning brain and autonomic nerves including vagus by Bailey & Bremer,[7)] Darrow etc.[8)]

As I studied the action potentials of brain, (E.E.G.) as related to those of autonomic nerves, some results obtained thereby I shall report here.

Experimental

Method

34 rabbits and cats were employed, all were anesthetized with urethane (1 g. per kg. subcutaneously). N. sympathicus and vagal nerve were both severed and thoroughly separated about 3 cm. long from the surrounding soft tissues and each was put on a pair of electrodes. In such procedures I took special care not to pull the nerves strongly. E.E.G. was led from frontal part of the skull of both sides with needle electrodes, common earth electrode being placed on the ear of the same side of the severed nerves. Action potentials of the brain and the autonomic nerves were led to amplifiers for E.E.G., specially constructed by Assist. Prof.

Watanabe and his collaborator Matsuo of the Department of Electrical Engineering of Tohoku University. The amplified potential changes were recorded photographically, and yet all simultaneously. Hypothalamic nuclei were located accurately by Hosley-Clark's apparatus and a small area (about 1 mm. in diameter) was electrocoagulated, the affected area was always confirmed afterwards by histological examination.

Results

I. Changes of action potential of cervical autonomic nerves and E.E.G. due to some drugs

1) About the judgement of records of action potential

Histogram of frequency of action potential waves of nerves and E.E.G. was constructed. The abscissa is scaled by logarithms of 39.2, 24, 15.5, 9.8, 6.2, 3.9, 2.4 per sec., and the ordinate is per cent of frequency of appearance among totals. Such histograms of E.E.G. and action potentials of autonomic nerves were compared each other before and after giving some drugs or stimulations of nerves. The changes amounting over than three times of mean error of histogram was decided as significant; in addition to this, changes in amplitude were also taken into consideration for the final judgement as a whole.

2) Spontaneous action potential of autonomic nerve at neck (Figs. 1, 2. Table I)

Amplitude of spontaneous action potential of sympathetic nerve is several to 15μV., and its histogram of frequency is shown in Fig. 1. The

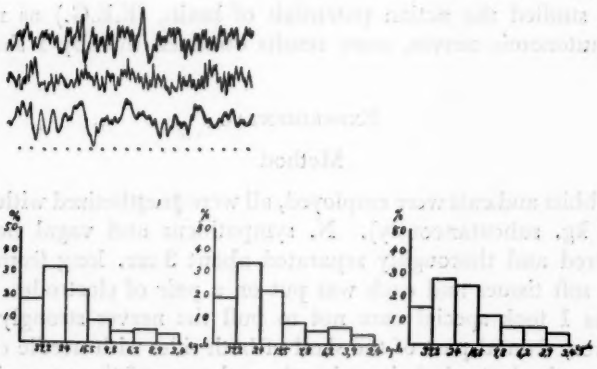

Fig. 1. Spontaneous action potential and its histogram of frequency. From the top downward action potential of sympathetic nerve, vagal nerve and E.E.G. Time: 1/100 second.

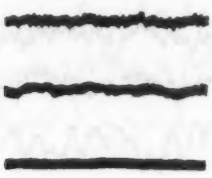

Fig. 2. Action potentials of the sympathetic (upper) and vagal nerve (middle) and E.E.G. (lower) when short-circuit is established (see explanation in text).

TABLE I

Frequency of Occurrence (in % of total number) of Action Potentials of Various Periods (expressed in cycles per second)

Cycle per second	Sympathetic nerve		Vagal nerve		E.E.G.	
39.2	18.9	3.1	20.4	2.4	16.6	2.7
24.0	33.6	2.2	35.0	1.5	28.7	1.9
15·5	23.2	2.5	23.3	1.2	26.3	1.3
9.8	9.9	1.2	9.1	1.1	13.1	1.0
6.2	5.6	0.6	5.7	0.9	7.4	0.8
3.9	6·5	0.8	6.5	1.0	7.6	0.9
2.4	4.8	1.1	4.1	0.7	5.2	0.5

peak of greatest occurrence is found between 39.2 per sec. and 24 per sec. In Table I, will be found arithmetical mean and mean error of each part of this histogram of frequency. An example of action potential led from vagal nerve is shown in Fig. 1, its amplitude reaches several to 15μV. and the peak of histogram of frequency is between 39.2 per sec. and 24 per sec. This potential change disappears (as shown in Fig. 2) when a pair of electrodes applied to nerve short-circuited, so that the potential change led from the nerve is obviously the potential change of nerve itself.

3) Potential change to adrenalin (Fig. 3)

5 rabbits were employed. 0.025 mg. of adrenalin pro kg. in 1 cc. of 0.9% of natrium chloride solution was injected in an ear-vein and in a few minutes potential changes were photographed. The wave, as in Fig. 3 was observed; though its amplitude was not changed evidently, its frequency decreased in the fast wave from 39.2 to 24 per sec. in all cases. Changes of potential of vagal nerve were not constant. In E.E.G. fast wave (24–15.5 per sec.) decreased in all cases and also intermediate wave (9.8–6.3 per sec.), increased, however, slow wave (3.9–2.4 per sec.) in 3 cases.

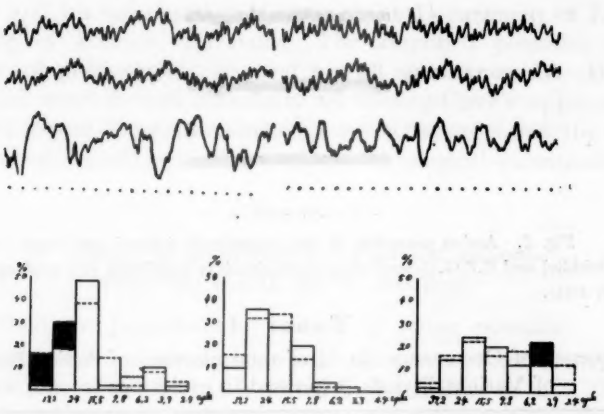

Fig. 3. Potential change due to adrenalin injection and histogram of frequency. From the top potential change of sympathetic nerve, vagal nerve and E.E.G. The left; before adrenalin injection. The right; after adrenalin injection. Histogram after adrenalin injection is shown with dotted line.

4) Potential changes to acetylcholine and metrazol (Fig. 4 & 5)

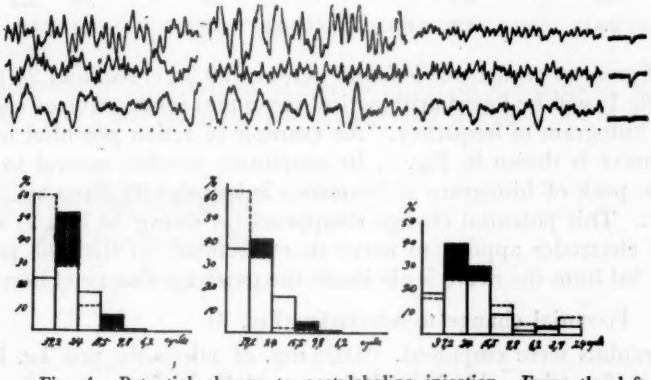

Fig. 4. Potential change to acetylcholine injection. From the left, before injection, acetylcholine injection and T.E.A.B. injection. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after acetylcholine injection is shown with dotted line.

0.05 mg. of acetylcholine or 0.1 cc. of metrazol in 1 cc. of 0.9% of natrium chloride solution were injected in an ear-vein in 4 cases and in a few minutes the potential changes were photographed. Convulsion did not occur. Amplitude of potential of both nerves turns into twice

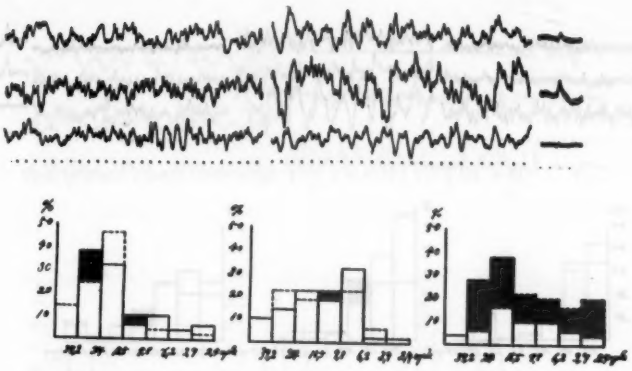

Fig. 5. Potential change due to metrazol injection. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after metrazol injection is shown with dotted line.

the control size. Fast wave (39.2–24 per sec.) of sympathetic nerve decreased and intermediate wave (15.5–9.8 per sec.) increased in 3 cases. In the vagal nerve, fast wave (39.2–24 per sec.) and intermediate wave (15.5–9.8 per sec.) decreased in 3 cases, in E.E.G. fast wave (39.2–24 per sec.) decreased, intermediate wave (24–15.5 per sec.) and slow wave (6.2-3.9 per sec.) increased in 3 cases.

Due to metrazol, change of frequency of sympathetic nerve was as follows; fast wave (39.2–24 per sec.) decreased in 3 cases and intermediate wave (15.5–9.8 per sec.) in all. In vagal nerve intermediate wave (15.5-9.8 per sec.) increased in 3 cases, fast wave (39.2–15.5 per sec.) of E.E.G. decreased in all cases, and intermediate wave (15.5–9.8 per sec.) in 3 cases, intermediate wave of 9.8–6.2 per sec. and slow wave (6.2–2.4 per sec.) increased in all cases. When 40 mg. of T.E.A.B. was injected into a vein, its amplitude decreased markedly.

5) Potential change due to pilocarpine (Fig. 6)

When 1 mg. pro kg. of pilocarpine in 0.9% of natrium chloride solution was injected into vein, amplitude usually increased markedly and especially in E.E.G. Change of frequency of sympathetic nerve potential was not constant, but intermediate wave (15.5–9.8 per sec.) of vagal nerve and also in E.E.G. increased in all cases. When 1 mg. of atropine pro kg. was given intraveneously at this time, the change due to pilocarpine disappeared instantaneously, and the potential returned to the state before pilocarpine-injection.

Results in this section above may be summarized as follows.

1. In action potential of sympathetic nerve, intermediate wave

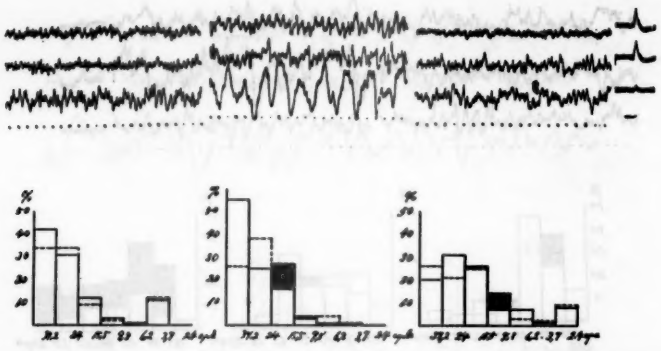

Fig. 6. Potetial change due to pilocarpine and atropine injection. From the left, before injection, pilocarpine injection and atropine injection. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after pilocarpine injection is shown with dotted line.

(10–15μV.) and fast wave (several μV.) were superimposed on slow wave (20–30μV.)

2. Potential changes of sympathetic nerve to adrenalin, metrazol and acetylcholine, that stimulate sympathetic nerve, are divided into two sorts. *The one:* fast wave (39.2–24 per sec.) decreases and there is no evident change in amplitude, in E.E.G. at the same time slow wave (6.2–3.9 per sec.) decreases. Such a change is observed to adrenalin injection. *The other:* fast wave (39.2–24 per sec.) and intermediate wave (15.5–9.8 per sec.) decrease. With markedly increased amplitude and in E.E.G. slow wave (6.2–3.9 per sec.) increases with high potential. Such a change is observed at acetylcholine and metrazol injection.

3. Changes in action potential of vagal nerve due to pilocarpine are an increase in amplitude and increase of intermediate wave (15.5-9.8 per sec.); in E.E.G. there are similar changes.

II. Potential change of autonomic nerves and E.E.G. when hypothalamic nuclei were electrocoagulated

Cats were used being anesthetized with urethane (1 g. per kg.), and potential change of autonomic nerve and E.E.G. were led as above described. To destroy hypothalamic nuclei an electrode was inserted in hypothalamus by means of Hosley-Clark's apparatus, and electrocoagulation (Phase of 1 mm. in diameter) was performed. After the experiment had been finished, the brain was removed, fixed and stained for accurate identification of the destroyed parts.

1) Potential change of autonomic nerves and E.E.G. when medial

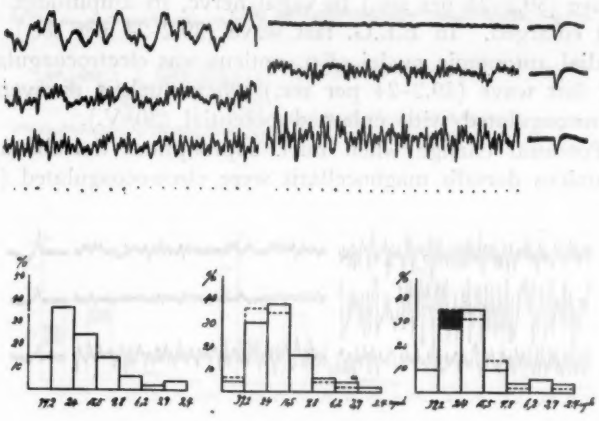

Fig. 7. Potential change due to electrical coagulation of anterior medial part of n. opticus. From the left, before coagulation and after coagulation. From the top, action potential of sympathetic nerve, vagal nerve and E.E.G. Histogram after coagulation is shown with dotted line.

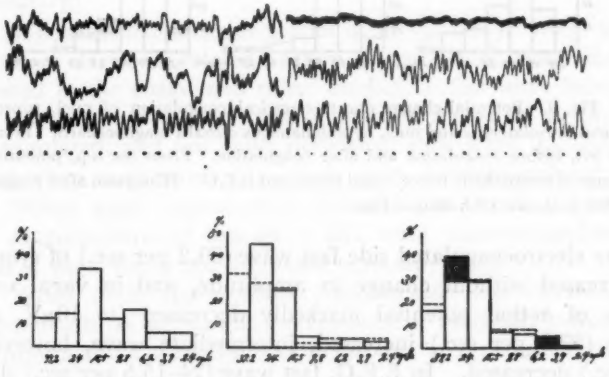

Fig. 8. Potential change due to electrical coagulation of nucl. paraventricularis. From the left, before coagulation and after coagulation. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after coagulation is shown with dotted line.

anterior nuclei of n. opticus were destroyed (Fig. 7 & 8)

Potential change of autonomic nerves following the electrocoagulation was more evident homolaterally in sympathetic nerve than in vagal nerve. As shown in Fig. 7, potential change was an almost complete disappearance of action potential in sympathetic nerve, and an increase

of fast wave (39.2–24 per sec.) in vagal nerve, its amplitudes, however, being not enlarged. In E.E.G. fast wave (39.2–24 per sec.) increased when medial autonomic nuclei of n. opticus was electrocoagulated, and decreased fast wave (39.2–24 per sec.) when nucleus paraventricularis was electrocoagulated with enlarged potential (30μV.).

2) Potential change when nucl. supraopticus dorsalis and nucl. hypothalamicus dorsalis magnocellaris were electrocoagulated (Fig. 9)

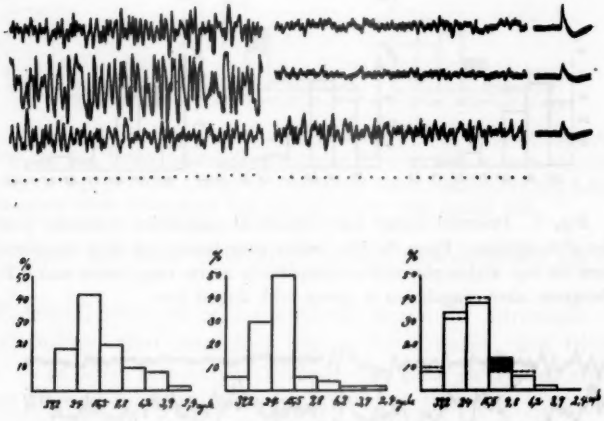

Fig. 9. Potential change due to electrical coagulation of nucl. supraopticus dorsolateralis and nucl. hypothalamicus dorsalis magnocellaris. From the left, before coagulation and after coagulation. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after coagulation is shown with dotted line.

In the electrocoagulated side fast wave (39.2 per sec.) of sympathetic nerve increased without change in amplitude, and in vagal nerve the amplitude of action potential markedly decreased (to 10μV. or less), fast wave (39.2 per sec.) increased, intermediate wave, however, (9.8-6.2 per sec.) decreased. In E.E.G. fast wave (24–15.5 per sec.) decreased and intermediate wave (9.8–6.2 per sec.) increased without any potential change.

3) Potential change when nucl. supraopticus dorsolateralis was electrocoagulated (Fig. 10)

In the electrocoagulated side potential of sympathetic nerve decreased, vagal nerve potential decreased also so markedly (to less than several μV.) that its frequency became hardly measurable. In E.E.G. intermediate wave (15.5–9.8 per sec.) decreased without any potential change.

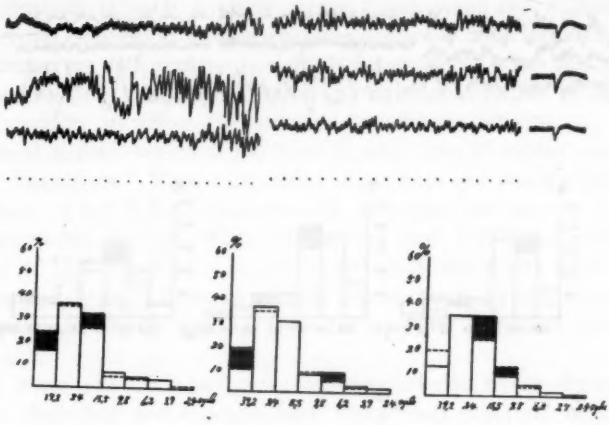

Fig. 10. Potential change due to electrical coagulation of nucl. supraopticus dorsolateralis. From the left, before coagulation and after coagulation. From the top, potential change of sympathetic nerve, vagal nerve, and E.E.G. Histolgram after coagulation is shown with dotted line.

Summary:

1. When medial anterior nuclei of n. opticus or nucl. paraventricularis were electrocoagulated, action potentials of sympathetic nerve disappeared in the coagulated side. In E.E.G. fast wave increased with increase in amplitude when medial anterior nuclei of n. opticus were electrocoagulated, and fast wave decreased, slow wave (6.2–3.9 per sec.) increased when nucl. paraventricularis were electrocoagulated.

2. When nucl. supraopticus dorsalis and nucl. hypothalamicus dorsalis magnocellaris of the other side were electrocoagulated, action potential of vagal nerve got decreased especially.

3. When nucl. supraopticus dorsolateralis was electrocoagulated, action potentials on autonomic nerves became markedly reduced and in E.E.G. intermediate wave increased without any potential change.

III. Potential change of cut autonomic nerves and E.E.G. when autonomic nerves were cut at neck (Fig. 11)

Autonomic nerves were cut in one side, and at both cut ends were led its action potentials along with E.E.G. At the central stump the amplitude of potential of both the sympathetic and vagal nerve got decreased. In the sympathetic nerve, the fast wave (39.2–24 per sec.) decreased, and the intermediate wave (9.8–6.2 per sec.) increased, while in the vagal nerve fast wave (24–15.5 per sec.) decreased, and the intermediate wave

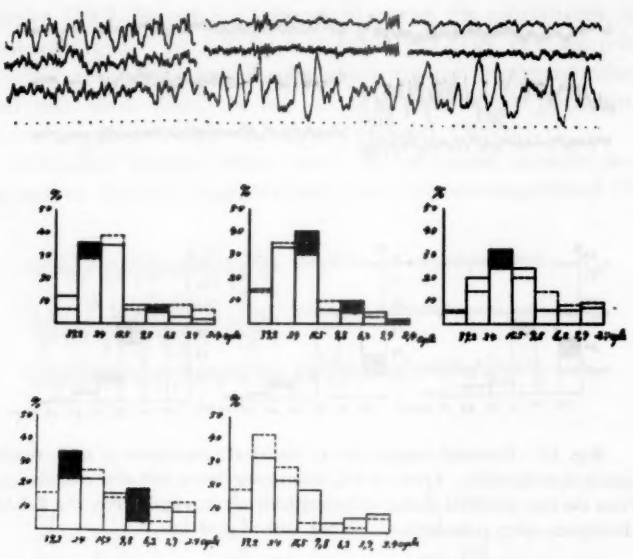

Fig. 11. Potential change at cut end of autonomic nerves. From the left, before cuting, after cuting at central end and at peripheral end. From the top, potential change of sympathetic nerve, vagal nerve and E.E.G. Histogram after cuting of nerves is shown with dotted line, the top shown the central cut end and the bottum the peripheral cut end.

(9.8–6.2 per sec.) increased. In E.E.G. the potential increased markedly. At the distal cut end the amplitude decreased evidently both in the sympathetic and vagus nerves. As to its frequency the fast wave (39.2–24 per sec.) of sympathetic nerve decreased, and the intermediate wave (9.8-6.2 per sec.) increased in all instances, but no constant potential changes were observed in the vagal nerve.

In short, when autonomic nerves were cut and potential changes were led at both cut ends, amplitudes of potential of sympathetic nerve as well as frequency of the fast wave decreased and frequency of the intermediate wave increased.

Discussion

Action potentials of peripheral nerves were studied by Erlanger, Gasser, Bishop and Adrian,[10] followed by Detlev & Frank,[11] Tasaki & Fujita[12] Bullock[13] and Young.[14] These authors all investigated chiefly of spike potentials of nerves. With E.E.G. apparatus we observed the waves of 39.2–24 per sec. in the autonomic nerves of cervical region. The

action potentials of such a whole nerve trunk were not studied so far. Such potential changes as I observed were definitely those of the autonomic nerves themselves, because the potentials disappeared when two electrodes were shortcircuited, and they changed characteristically due to some drugs known especially to affect the autonomic nervous activity.

Cortical E.E.G. which is led from frontal area of rabbit was studied by Ito & Kitamura. They said that E.E.G. does not change particularly by location. I led E.E.G. from frontal area that was said to be related to the autonomic nerve. While Davis says that E.E.G. of rabbit has a frequency of 13.5–8 per sec., in our experiment fast waves were observed more abundantly in 2 cases of rabbits anesthetized by urethane than in normal animals. Such finding may be ascribed to effect of urethane injection.

Fast wave (24–15.5 per sec.) in sympathetic nerve decreased by adrenalin injection and in vagal nerve there was observed no particular change; in E.E.G. slow wave of 6.2–3.9 per sec. increased with slightly diminished amplitude. Davis[1] says that α and β waves increase with increased amplitude due to sensory stimulation. Bartiley & Hembecker[15] reported the amplitude diminished in some conditions, and Lennox & Gibbs[5] say that the influence of adrenalin to E.E.G. is insignificant. I consider that adrenalin plays influences upon E.E.G. through a reflex from its periphery, because the potential change of autonomic nerves was observed at both cut ends at the neck.

In single fibre preparations spike potentials were observed due to acetylcholine (Bullock,[13] Young[14]). The action potentials of autonomic nerve trunk at neck were different from it and rather large potential waves were seen in the sympathetic nerve with a decrease of fast waves and the intermediate wave. In the vagal nerve, the change in frequency was the same but its amplitude was not changed. The change of E.E.G. due to acetylcholine has been discussed as "acetylcholine discharge" by many authors, Miller, Stavraky & Woonton,[16] Foerster,[17] Bonnet & Bremer,[18] Chatfied Dempsy.[19] My results were almost the same as these authors', that is, slow waves of high potential and fast wave of 24-15.5 per sec, were increased.

Change of action potential of the sympathetic nerve due to metrazol was almost the same as due to acetylcholine injection, but its amplitude was less than the amplitude in cases of acetylcholine injection. In vagal nerve the intermediate wave increased with enlarged amplitude and in E.E.G. the intermediate wave and the slow wave increased. That is, potential change of sympathetic nerve and E.E.G. due to adrenalin, acetylcholine and metrazol (all these drugs stimulate the sympathetic nerve), is divided into two sorts, one is such that in the sympathetic nerve

fast wave and slow wave of E.E.G. decreases, another is such that the fast wave and the intermediate wave decreases in sympathetic nerve and slow wave of E.E.G. increases.

Potential change of sympathetic nerve to pilocarpine is not significant and in vagal nerve fast wave increases, in E.E.G. intermediate wave and slow wave of high potential increase markedly. The same result was reported by Darrow & Pathman,[10] Bailey & Bremer,[7] Darrow, Green, Davis & Garol.[8] When vagal nerve is stimulated, its action potential of the intermediate wave increases and the same change is observed in E.E.G. Thus metrazol may lead to stimulation of the vagal nerve because these changes are observed also when metrazol is given:

Gerard, Marshall & Saul,[21] Kornmüller[22] and Kennard[23] reported of the correlation between hypothalamus and E.E.G.: the former authors say that the hypothalamus has no relation to E.E.G. and the latter authors say that the hypothalamus has some influences on the cortical E.E.G. We observed some changes in frequency and amplitude when one of these nuclei, that is, medial anterior nucl. of n. opticus, nucl. supraopticus dorsolateralis, nucl. hypothalamicus dorsalis magnocellaris and nucl. paraventricularis, was electrocoagulated. These nuclei show influences on cortical E.E.G. Moreover, medial anterior nucl. of n. opticus, nucl. paraventricularis and nucl. hypothalamicus dorsalis magnocellaris are closely connected with action potential of the cervical sympathetic nerve, and nucl. supraopticus dorsolateralis with action potential of both sympathetic and vagal nerves at neck.

Conclusion

In concluding my report I would emphasize the following facts:

1. Action potential of sympathetic and vagal nerve at neck is 39.2-2.4 per sec. in freqnecy, the majority being 39.2–15.5 per sec.

2. Change of action potential of sympathetic nerve at neck due to adrenalin is a decrease in fast wave (39.2–15.5 per sec.) and in E.E.G. a decrease in slow wave (6.2–3.9 per sec.).

3. Of the action potentials of sympathetic nerve at neck the fast wave (39.2–24 per sec.)' and the intermediate wave (15.5–9.8 per sec.) decrease and in E.E.G. the slow wave (6.2–3.9 per sec.) increases due to acetylcholine. As to the action potential of sympathetic nerve to metrazol the intermediate wave (15.5–9.8 per sec.) and slow wave (6.2–3.9 per sec.) decrease, in the vagal nerve the intermediate wave (15.5–9.8 per sec.) and in E.E.G. the intermediate wave (15.5–9.8 per sec.) and slow wave 6.2–3.9 per sec.) increase.

4. Concerning the action potential of vagal nerve at neck, pilocarpine increases the intermediate wave (15.5–9.8 per sec.). In E.E.G.

the intermediate wave (15.5–9.8 per sec.) with high potential increases in frequency.

5. Damage of medial anterior nucl. of n. opticus, nucl. paraventricularis, nucl. supraopticus dorsalis and nucl. hypothalamicus dorsalis magnocellaris affects the cortical E.E.G., lesion of the former two results in a disappearance or marked decrease of action potential of the sympathetic nerve, lesion of the latter two results in changes in action potentials of sympathetic and vagal nerve.

6. Potential changes are observed at both cut ends of both the sympathetic and the vagal nerve when they are cut at the neck.

I express my appreciation to Prof. S. -T. Katsura and Prof. K. Motokawa whose cordial guidance made me carry out the present investigation, and to Prof. K. Matsuda for his assistance in Preparation of this manuscript.

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Experimental Study of Action Potential of Thyroid Gland

By

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Studies of action potential of thyroid gland were first reported by Cannon & Cattel,[1] who observed action potential of thyroid gland of cat following adrenalin injection or electrical stimulation of sympathetic nerve; later on, Hasama[2] reported of the changes in action potential of thyroid gland of rabbit by some drugs or electrical stimulation of cervical sympathetic nerve. The aim of my study, the result of which is reported here, is to observe the functions of the thyroid gland of rabbits as revealed by its action potentials both in resting and excited conditions produced by some drugs or electrical stimulation of autonomic nerves.

Experimental

Method

Adult rabbits of about 2 kg. by weight were fixed on the back and anesthetized with urethane (1 g. pro kg.), at times combined with ether or chloroform. Care was taken not to use pregnant rabbits. After the anterior cervical region was dissected about five cm. long, the thyroid gland was exposed carefully not to be injured and tracheal canula was inserted. The operative area was adequately warmed by an electric lamp and special care was taken to avoid local drying.

For registration of action potentials of the thyroid gland; two galvanometers were used: one was R-type, internal resistance=300Ω, current sensitivity=2.4×10^{-8} A., voltage sensitivity=7.2×10^{-7}V., period 4 sec., another was 30Ω, 2.4×10^{-9} A., 2.5×10^{-7}V., period 9 sec., respectively, electrodes used were nonpolarizable zinc-zinc sulfate-cotton thread electrodes soaked with 0.9% solution of natr. chloride, one being placed on the thyroid gland, the other on the adjacent subcutaneous fat tissue.

The recording camera was placed so distant that the deflexion was

60 mm. by 1 mV. The action potential was usually recorded continuously for forty to fifty minutes. The action potentials were followed at first in quiet and resting condition, then its changes following the electrical stimulation of the sympathetic or the vagal nerve and at last effects of drugs, such as adrenalin, pilocarpine, nicotine, strychnine, metrazol and iodine were observed. The changes were studied in two aspects: (1) the latency in reaction and initial phase of change and (2) the later phase of reaction.

Results

1. Spontaneous action potential of the thyroid gland (Fig. 1 a, b and Table I)

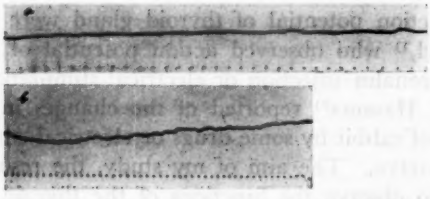

Fig. 1. Spontaneous action potential of the thyroid gland, rabbit. Time mark, a: five seconds, b: thirty seconds.

TABLE I

Period and Amplitudes of Spontaneous Action Potentials of the Thyroid Gland of Rabbits

Upper figure in each is period, lower figure is amplitude. Three columbs signify the values of the three successive waves observed.

Case No.	1st Wave	2nd Wave	3rd Wave
1	7′30″ 0.11mV.	11′ 0.19mV.	6′ 0.07mV.
2	6′30″ 0.09mV.	10′30″ 0.09mV.	12′ 0.09mV.
3	7′ 0.13mV.	11′ 0.13mV.	7′30″ 0.09mV.
4	5′30″ 0.07mV.	7′30″ 0.1mV.	8′30″ 0.1mV.
5	7′ 0.07mV.	6′ 0.07mV.	10′ 0.07mV.
Average		8′ 0.10mV.	

This was examined in five animals. Even without any stimulation there were occasionally observed small waves of 0.05 mV. in amplitude

and 30 sec. to 1 min. in period. Both the ascending and descending limbs of this wave were gentle slopes and symmetrical and the peak was rather flat. Such a spontaneous wave seemed to appear without any relation to the breathing (Fig. 1 a.).

Besides the potential change mentioned above, during continous observations extending to about one hour, another type of wave was seen, that is a wave of 6 to 12 minutes' period (8 min. in average) and 0.07 to 0.13 mV. in amplitude (0.1 mV. in average) with gentle slopes of ascending and descending limbs symmetrical in shape, and superimposed by the small waves described above. Such a wave recurred about 2.5 times in one hour. In almost all cases the base line slowly changed more or less, except in one case where the base line did not changed at all.

2. Reaction changes of the action potential to electrical stimulation of the sympathetic nerve (Fig. 2a, b and Table II)

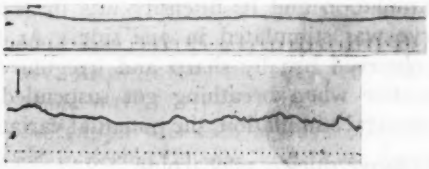

Fig. 2. Change in action potential to electrical stimulation of sympathetic nerve in rabbit. Arrow shows stimulation, time mark, a: five seconds, b: thirty seconds.

TABLE II

Effects of Electrical Stimulation of Sympathetic Nerve upon the Thyroid Action Potential

Case No.	Latent Time	1st Wave		2nd Wave	
		Period	Amplitude	Period	Amplitude
1	5″	2′30″	0.14mV.	15′	0.10mV.
2	6″	2′15″	0.15mV.	4′	0.08mV.
3	6″	2′20″	0.12mV.	15′	0.12mV.
4	7″	1′40″	0.10mV.	17′	0.20mV.
5	7″	1′55″	0.14mV.	16′	0.21mV.
Average	6.2″	2′8″	0.13mV.	13′15″	0.14mV.

Induction current with Wagner's hammer (Coil distance=8 cm.) was used as stimulus. This was applied for thirty sec. to the cervical sympathetic nerve at the site of about three cm. distant from the thyroid gland. After 6.2 sec. of latent period there was observed a reaction wave of action potential of 1 min. 55 sec. to 2 min. 30 sec. in period (8 sec. in

average) and 0.10 to 0.15 mV. in amplitude (0.13 mV. in average). This was a gentle wave consisted of ascending limb, plateau and descending limb. The ascending limb was more sharp than the descending, the latter often did not return to the pre-excitation level. In the succeeding period potential showed almost no change for a few minutes. But thereafter appeared a slow wave of 4 to 17 min. in period (13 min. and 15 sec. in average), 0.08 to 0.21 mV. in amplitude (0.14 mV. in average), its ascending and descending phases being symmetrical. Though superimposed with a few small wavelets of less than 0.01 mV. in amplitude, this appeared as a whole a gentle and long wave. In forty minutes these changes usually disappeared and the potential returned to the base line. Such was a general tendency in all five cases (Table II).

3. Potential change due to electrical stimulation of the cervical vagus

Method of stimulation and its intensity was the same as above described. The nerve was stimulated in one side. At the beginning of stimulation were observed usually sharp and irregular waves for a few seconds, but thereafter when breathing got suspended and pulse rate slowed down due to vagal stimulation, the potential variations disappeared.

4. Potential change due to pilocarpine

Pilocarpine 0.01 g. pro kg. in 0.5 cc. of 0.9% saline solution was injected into an ear-vein. No remarkable potential change of action potential was observed.

5. Potential change due to adrenalin (Fig. 3 a, b and Table III)

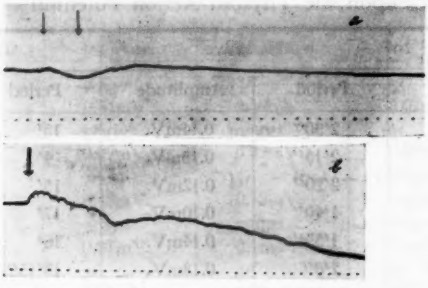

Fig. 3. Action potential due to adrenalin injection. Arrow shows injection, time mark, *a*: five seconds, *b*: thirty seconds.

Andrenalin 0.2 mg. pro kg. in 0.5 cc. of 0.9% saline solution was injected into an ear-vein. After 10.6 seconds of latent period there was observed a wave of 2 to 3 min. in period (2 min. and 28 sec. in average)

TABLE III

Effects of Adrenalin Injection upon the Thyroid Action Potential of Rabbits

Case No.	Latent Time	1st Wave		2nd Wave		3rd Wave	
		Period	Amplitude	Period	Amplitude	Period	Amplitude
1	8″	1′40″	0.22mV.	5′	0.1mV.	9′30″	0.18mV.
2	10″	2′15″	0.24mV.	5′30″	0.1mV.	9′30″	0.13mV.
3	11″	3′	0.20mV.	3′	0.08mV.	17′	0.16mV.
4	12″	2′50″	0.14mV.	4′30″	0.13mV.	11′	0.10mV.
5	12″	2′35″	0.30mV.	6′30″	0.08mV.	8′30″	0.10mV.
Average	10.6″	2′28″	0.22mV.	4′54″	0.10mV.	11′12″	0.13mV.

and 0.14 to 0.30 mV. in amplitude (0.22 mV. in average). The shape of this wave was the same as that by electrical stimulation of sympathetic nerve. Then in a few minutes another wave appeared: 3 min. to 6 min. and 30 sec. in period (4 min. and 54 sec. in average) and 0.08 to 0.13 mV. in amplitude (0.1 mV. in average); the peak was situated rather before the middle of the whole wave.

In a longer observation was seen a wave of gentle slope of 8 min. to 17 min. and 30 sec. in period (11 min. and 12 sec. in average) and about 0.12 mV. in amplitude; the peak of it was found in advance of the middle of the wave and was superimposed with many small waves of one minute's period. Some changes in base line of potential were observed in all 10 cases.

6. Potential change by nicotine (Fig. 4 a, b and Table IV)

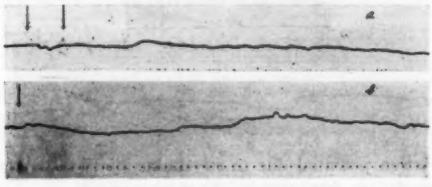

Fig. 4. Action potential due to nicotine injection. Arrow shows injection, time mark, *a*: five seconds; *b*: thirty seconds.

Nicotine 0.1–0.2 mg. pro kg. in 0.5 cc. of 0.9% solution of natr. chloride was injected into an ear-vein. Convulsion did not occur. Latent time was 11 sec., period was 3 to 5 min. (4.2 min. in average) the peak was 0.11 mV. in amplitude.

The shape of the wave was flat as a whole and superimposed with small waves, intermediate phase being longer than ascending and des-

TABLE IV

Effects of Nicotine upon the Thyroid Action Potential of Rabbits

Case No.	Latent Time	1st Wave		2nd Wave		3rd Wave	
		Period	Amplitude	Period	Amplitude	Period	Amplitude
1	7″	3′	0.10mV.	11′	0.13mV.	12′	0.17mV.
2	10″	5′	0.13mV.	16′	0.33mV.		
3	11″	4′	0.10mV.	4′	0.15mV.	8′	0.20mV.
4	12″	4′	0.10mV.	6′	0.10mV.	10′	0.10mV.
5	15″	5′	0.12mV.	15′	0.18mV.		
Average	11″	4′02	0.11mV.	10′24″	0.18mV.	10′	0.16mV.

cending ones.

At one hour's observation there were observed other two waves. One wave followed the above mentioned wave, and its ascending and descending phases were gently sloping and symmetrical, and its period was 4 to 16 min. (10 min. 24 sec. in average). The amplitude was 0.1 to 0.33 mV. (0.18 mV. in average), the peak was at the middle of the wave. This wave was followed by the third, also gentle wave which was 10 to 12 min. in period (10 min. in average) and 0.1 to 0.20 mV. in amplitude. The intermediate phase was longest and superimposed with many small waves of one minute in period.

7. Potential change by strychnine (Fig. 5 a & b, Table V)

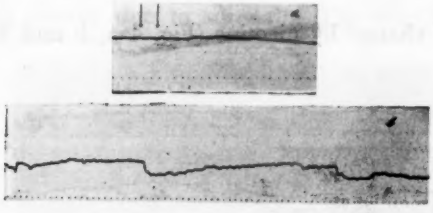

Fig. 5. Action potential due to strychnine injection. Arrow shows injection, time mark, *a*: five seconds, *b*: thirty seconds.

Strychnine, 0.05 mg. pro kg. in 0.5 cc. of 0.9% solution of natr. chloride was injected into an ear-vein; convulsion did not occur. After the latent time of 6 seconds was observed a wave of 1 to 2 min. in period (1 min. and 36 sec. in average) and of 0.05 to 0.09 mV. in amplitude (0.07 mV. in average). The shape of this wave was as a whole flat, and the ascending phase was more sharp than the descending and the peak was near to the ascending phase. This wave was followed by another wave

TABLE V

Effects of Strychnine Injection upon the Thyroid Action Potential of Rabbits

Case No.	Latent Time	1st Wave		2nd Wave		3rd Wave	
		Period	Amplitude	Period	Amplitude	Period	Amplitude
1	4″	1′30″	0.05mV.	7′30″	0.08mV.	16′	0.10mV.
2	5″	1′	0.08mV.	3′	0.14mV.	7′	0.11mV.
3	5″	2′	0.06mV.	7′	0.08mV.	21′	0.20mV.
4	6″	2′	0.08mV.	3′30″	0.08mV.	16′	0.10mV.
5	10″	2′	0.09mV.	6′30″	0.11mV.	16′	0.16mV.
Average	6″	1′36″	0.07mV.	5′30″	0.10mV.	14′12″	0.13mV.

of 3 min. to 7 min. and 30 sec. in period (5 min. and 30 sec. in average) 0.08 to 0.14 mV. in amplitude (0.10 mV. in average) and more flat than the former. At one hour's observation a long wave appeared, the ascending phase of which lasted about one min. to go over into the peak, followed by the descending phase of gentle slope. The period of this wave was 7 to 21 min. (4.12 min. in average), and its amplitude was 0.1 to 0.20 mV. (0.13 mV. in average). Small waves of potential of about one minute's duration were superimposed upon the wave as usual.

8. Potential change by metrazol (Fig. 6 a & b, Table VI)

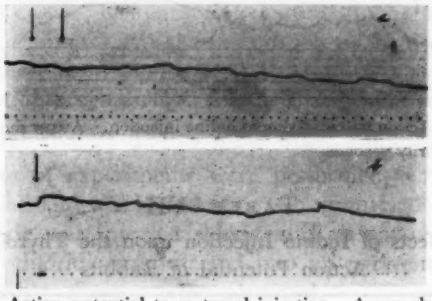

Fig. 6. Action potential to metrazol injection. Arrow shows injection, time mark, *a*: five seconds, *b*: thirty seconds.

Metrazol of 0.3 cc. pro kg. in 0.5 cc. of 0.9% solution of natr. chloride was injected into an ear-vein. Experiments performed in 10 animals, convulsion was not observed. After a latent time of 6 seconds, there appeared a wave. The period was 14 min. 12 sec. The ascending phase of this wave was sharp, and before long its potential reached to the highest level (0.17 mV.) and step by step changed itself into the descending phase with a gentle slope. Following this wave appeared another wave, the

TABLE VI

Effects of Metrazol Injection upon the Thyroid Action Potential of Rabbits

Case No.	Latent Time	1st Wave		2nd Wave	
		Period	Amplitude	Period	Amplitude
1	3″	11′	0.17mV.	8′	0.15mV.
2	5″	16′	0.24mV.	5′	0.10mV.
3	5″	16′	0.13mV.	6′	0.07mV.
4	7″	14′	0.18mV.	8′	0.18mV.
5	10″	14′	0.12mV.	5′	0.07mV.
Average	6″	14′12″	0.17mV.	6′12″	0.11mV.

peak of which was before its middle, its ascending and descending phase being both gentle slopes, and superimposed with small wavelets.

When 0.5 cc. of metrazol were injected, divided in a few parts during 30 min. and sympathetic nerve was electrically stimulated, the latent time was shortened into two seconds and the period extended to several minute; but the potential showed no marked changes.

9. Potential change by iodine (Fig. 7, Table VII)

Lugol's solution (Jod 1.0, Kal. jod. 2.0, Aq. dest. 27) was diluted

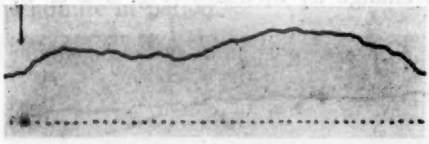

Fig. 7. Action potential due to iodine injection. Arrow shows injection, time mark, thrity seconds.

TABLE VII

Effects of Iodine Injection upon the Thyroid Action Potential of Rabbits

Case No.	Latent Time	1st Wave		2nd Wave		3rd Wave	
		Period	Amplitude	Period	Amplitude	Period	Amplitude
1	6″	10′	0.11mV.	6′	0.07mV.	20′	0.10mV.
2	8″	13′	0.19mV.	17′	0.14mV.	10′	0.10mV.
3	9″	2′	0.07mV.	20′	0.14mV.		
4	10″	11′	0.07mV.	20′	0.13mV.	12′	0.14mV.
5	12″	8′	0.18mV.	14′	0.28mV.	11′	0.11mV.
Average	8.8″	8′48″	0.12mV.	15′24″	0.15mV.	13′15″	0.12mV.

10 times with 0.9% solution of natr. chloride and one cc. of it was injected into an ear-vein in 10 cases. The latent time was 8.8 sec. The first wave was 2 to 13 min. in period (8 min. and 48 sec. in average) and 0.07 to 0.19 mV. in amplitude (0.12 mV. in average), and the ascending and descending phases were symmetrical each with a gentle slope, and the peak was found in the middle of the wave. In the following fifty minutes' observation two waves were observed, the one was 6 to 20 min. in period (15 min. and 24 sec. in average), 0.07 to 0.28 mV. in amplitude (0.15 mV. in average) and the shape of the wave was the same as the first wave. This wave was followed by the other, which was 11 to 20 min. in period (13 min. and 14 sec. in average) 0.10 to 0.14 mV. in amplitude, the shape being almost the same as the former. These 3 waves were all superimposed with small waves of potential of about one minute in period.

10. Potential change by ergotinine (Fig. 8)

Fig. 8. Action potential due to sympathetic nerve blockade. Arrow shows stimulation. Time mark, five seconds.

0.5 mg. in 5 cc. of 0.9% solution of natr. chloride was injected into an ear-vein in 9 cases. Changes of potential similar as above described were noted in two minutes. When in addition to the drug the stimulation of sympathetic nerve as in the former paragraph was given, any potential changes were not observed at least for the first 8 minutes in all of 9 cases.

11. Potential change due to some other stimulations

Several types of experiments were performed, viz.: carotid artery of one side was tied and was loosed after two minutes, 1 cc. of 1% solution of barium chloride was injected slowly into an ear-vein, 1 cc. of 0.9% solution of natr. chloride was injected into an ear-vein. In all of these cases no remarkable changes were observed.

Discussion and Summary

Dungern[3] observed spontaneous action potential of the kidney of rabbit, and Hasama[4] observed that of liver and thyroid gland. They said, such potential changes are small and of about one minute's period. We also observed the same potential change at thyroid gland of rabbit, which appeared occasionally and had not any special relation with respiration as Hasama pointed out. Influences of breathing on the

TABLE VIII

Characteristics of Thyroid Action Potential Waves in Normal as well as Excited Conditions of Rabbits

Upper figure of each set of values is period, lower figure is amplitude, both being averages.

	Latent time	1st Wave	2nd Wave	3rd Wave
Sympathetic nerve stimulation	6.2″	2′8″ 0.13mV.	13′15″ 0.14mV.	
Adrenalin	10.6″	0′28″ 0.22mV.	4′54″ 0.10mV.	11′12″ 0.13mV.
Nicotine	11″	4′02″ 0.11mV.	10′24″ 0.18mV.	10′ 0.16mV.
Strychnine	6″	1′36″ 0.07mV.	5′30″ 0.10mV.	14′12″ 0.13mV.
Metrazol	6″	14′12″ 0.17mV.	6′12″ 0.11mV.	
Iodine	8.8″	8′48″ 0.12mV.	15′24″ 0.15mV.	13′15″ 0.12mV.

Spontaneous . . . . . 8′
0.10mV.

thyroid potential, if ever present, should have been excluded by insertion of a tracheal canula; even in such circumstances some changes in potential occurred spontaneously. Dungern distinguished the respiratory potential change from the spontaneous potential change. In addition to these I noticed a new another wave (0.1 mV. in amplitude and 8 minutes in period) which was superimposed with the small potential changes, the so-called " spontaneous waves " by other authors. Both together, I think, constitute the spontaneous action potential of the thyroid gland.

When sympathetic nerve is stimulated electrically or when adrenalin is administered, an action potential of two to several minutes in period is observed. This change was reported already by Cannon & Cattel and Hasama. In my experiment, I confirmed their findings as to the latent time and the first wave; besides I found also the appearance of the second and the third waves which were not known up to the present. The period of the second or the third waves that follow the first wave is larger than the period of the first wave. As no remarkable change in potential was observed after the second and the third waves, these waves may be regarded as reaction changes to electrical stimulation of the sympathetic nerve or to adrenalin injection.

Potential change to nicotine was observed by Hasama, who said the wave was diphasic or triphasic and of a few minutes in period. As to the latent time and the first wave I agree with him. Moreover I discovered the second and the third waves as in the case of sympathetic

stimulation, but the period and amplitude of the later waves after nicotine injection were larger than in sympathetic stimulation.

Hasama said that the potential change to strychnine revealed itself as many waves with various periods and amplitudes, and that the bottoms of these waves were never higher than the initial potential; in my experiment the result was somewhat different, that is, the potential changes occurred not only in an initial few minutes but also afterwards in forty minutes, and such later waves, the second and third waves decreased in period and increased in amplitude. Latent time of action potential due to metrazol injection was not significantly different from the cases of other drugs, but remarkable was it, that in cases of metrazol the period of the first wave was longer than that of the second wave.

When metrazol was injected, divided in several parts, electrical stimulation of sympathetic nerve resulted in shortening of latent time and enlargement of the first wave, that is, the excitability of the gland seemed to be increased.

Potential change due to iodine was seen for more than 40 minutes. Of the first wave shortening of its period, of the second and third waves elongation of period was seen.

Where do these potential changes come from? There are three possiblities: the first, due to a change in blood flow in the thyroid gland, the second, due to contraction of smooth muscles of thyroid and the third, due to hyperfunction of thyroid. Even when one carotid artery is clamped and the blood flow to the thyroid is changed or when barium chloride is injected intravenously to make smooth muscles to contract, no particular potential change is observed, so that we may say the changes described above may be due to hyperfunction of the thyroid gland. In considering the fact that when sympathetic nerve block by ergotinine is produced, potential change is very faint or does not appear at all after various types of excitation, it seems to be justified that all the above mentioned stimulations affect the thyroid gland through the sympathetic nerve.

Conclusion

The action potential of thyroid gland of rabbit was studied in 74 cases, and the results obtained are as follows:

1. Spontaneous action potential appears as a wave of about 8 minutes in period and 0.1 mV. in amplitude. Superimposed upon this wave, some small wavelets of about one minute are noticed.

2. The reaction pattern of the action potential of thyroid is observed by electrical stimulation of sympathetic nerve, or by the action of adrenalin, nicotine, strychnine, metrazol and of iodine. These changes appear usually in thirty to forty minutes.

3. Secretory function of thyroid gland seems to be affected by sympathetic nerve as revealed by the changes in its action potential.

My thanks are due to Prof. S. -T. Katsura and Prof. K. Motokawa whose cordial guidance enabled me to perform the present study, and to Prof. K. Matsuda for his assistance in preparation of the manuscript.

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Properties of Retinal Induction in the Human Eye

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Introduction

Visual contrast or induction is a familiar phenomenon, but its mechanism is quite obscure. It must be due to the lack of adequate methods to make this phenomenon accessible to any quantitative measurements.

In the previous papers[1)2)] it was shown by the author that the method of electro-stimulation, which had proved to be a serviceable means for analyzing retinal color processes, could be used for the study of retinal induction as well. Some properties of retinal induction were revealed by this method, and the importance of retinal induction as a physiological event underlying a variety of psychological phenomena such as contrast, optical illusion, "gestalt", etc. was emphasized based upon the data obtained by this method.

However, physiological data concerning this phenomenon are yet so scant that we have yet no clue to the mechanism of induction. What we can do at present is to provide experimental materials as many as possible and to try to find any regularities and interrelations existing among them.

Experimental

Method

In the preceding paper,[2)] the terms "direct induction" and "indirect induction" were introduced in order to distinguish physiological effects from psychological ones such as successive and simultaneous induction. By direct induction we mean a physiological after-effect due to an illumination which is proved within the area pre-illuminated, and by indirect induction a physiological effect simultaneously induced around a retinal image is meant. In the present investigation these terminologies

will be used, and the effect of light will be expressed in terms of ζ which is defined by the formula: $\zeta=100\ (E-E_0)/E_0$, where E and E_0 denote electrical sensitivities measured with and without pre-illumination.

As inducing light exclusively sodium yellow light was used, for such strong monochromatic light was advantageous for producing strong retinal induction. To measure the direct induction caused by the sodium yellow light, the electrical excitability is measured at the moment 3 seconds after termination of white test-light which is preceded by the yellow inducing light. ζ-values determined in this way are generally higher than those for the white test-light alone. The difference of these two kinds of ζ-values is termed " contrast effect " and serves as a quantitative measure for retinal induction.

In order to measure indirect induction, the subsequent white test-light is made to fall on an adjacent part to the retinal area pre-illuminated by the yellow light and excitability measurements are done 1.5 sec. instead of 3 sec. after removal of the white light. As to the technique of electrostimulation of the eye the papers[3)4)] should be consulted.

The patches used for illumination consisted of a plate of ground glass and 2 sheets of black paper. An example is shown in Fig. 1. In this case the inducing figure is a half-ring (A), and the white test-light is a circular patch 2 mm. in diameter (B). The latter is to be presented

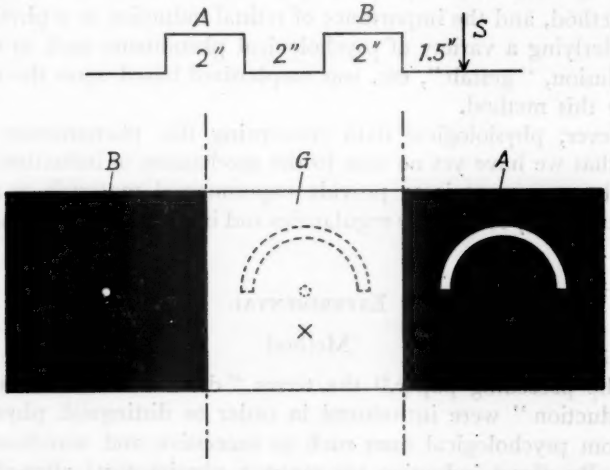

Fig. 1. Arrangement of patches. A: a sheet of black paper on which an inducing figure is cut (a half-ring in this case). B: a sheet of black paper with a circular opening of 2 mm. in diameter. G: ground glass. Order of presentation of patches is indicated on top.

at the center of the half-ring. When the inducing figure was to be presented, the paper A was laid upon the ground glass, and then this was illuminated by the yellow light from behind. To present the white test-light, the ground glass was illuminated by white light after the paper A had been replaced by the paper B. The operation of these patches A and B was done by the subject in the dark interval between successive illuminations. The distance between the patch and the subject's eye was always 30 cm.

Results

1. Dependence of retinal induction upon retinal area illuminated

In the previous experiment[1] it was established that the intensity of retinal induction expressed in terms of contrast effect is proportional to the logarithm of the intensity of inducing light, while it depends little on the intensity of the white test-light. The question now arises as to how it depends on the area illuminated.

In the following experiment, the direct induction caused by circular patches of varying diameters was measured, using white test-patches of the same size as the inducing ones. The data are shown in Table I. As

TABLE I

Illuminated Area and Intensity of Direct Induction

Diameter of patch in mm.	ζ for yellow alone	ζ for white alone	ζ for yellow-white	Contrast effect
2	24.5	19.5	28.5	9.0
4	30.0	25.0	45.5	20.5
8	46.0	41.0	61.3	20.3
16	48.5	43.5	64.0	20.5

can be seen in this table, ζ-values for the yellow patch alone or the white patch alone increase with their diameter. This is an expression of spatial summation of retinal excitation. The contrast effect also increases with the area so long as it is sufficiently small, but remains almost constant irrespective of the area illuminated when it is greater than 3 mm. It is to be noted that summation is seen with respect to retinal excitation or ζ-values for yellow or white illumination alone, whereas no summation is found with respect to induction or contrast effects.

Next, the dependence of indirect induction upon the area illuminated was studied, using rings of varying widths as inducing patches. The white test patch used was a circle of 2 mm. in diameter presented at the center of the ring (see Fig. 2). The data obtained are represented in

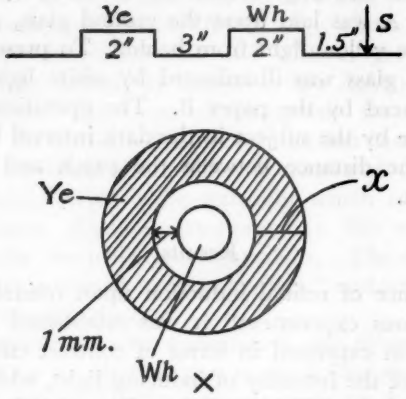

Fig. 2. Ye: yellow inducing patch. Wh: white test-patch. x: variable. Cross indicates point of fixation.

Table II. Contrast effects were found almost constant, indicating that indirect induction is independent of the width of the inducing ring. From this finding it follows that the indirect induction at the center of the ring

TABLE II

Illuminated Area and Intensity of Indirect Induction

Width of ring in Fig. 2	ζ for white alone	ζ for yellow-white	Contrast effect
1 mm.	26	46.8	20.8
2 "	"	46.0	20.0
4 "	"	45.2	19.2
8 "	"	46.0	20.0

was determined by the light falling within the innermost zone of about 1 mm. width of the ring, while the other part of the ring contributed nothing to the induction under consideration.

Another series of experiment was carried out with a rectangular patch 20 mm. long. The width of the rectangle was varied and the indirect induction at a point 3 mm. from the margin was measured. The data are shown in Table III, which indicates that the contrast effect is independent of the area of the rectangle when its width is greater than 2 mm. This finding also suggests that the area itself plays no important role in producing retinal induction. In view of the phenomenon of border contrast it is conceivable that the contour of an inducing figure plays a more prominent role in this regard, and the following experiment seems

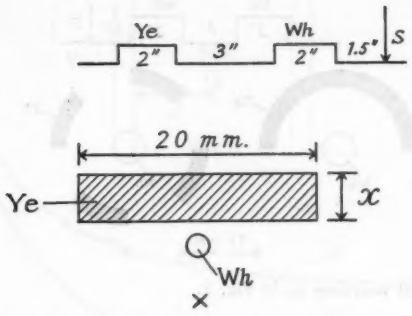

Fig. 3. All notations as in Fig. 2.

TABLE III

Illuminated Area and Intensity of Indirect Induction

Width in mm. of rectangle in Fig. 3	ζ for white alone	ζ for yellow-white	Contrast effect
1.0	21	31	10
2.0	"	42	21
3.5	"	43	22
4.0	"	43	22
8.0	"	42	21

to support this view. The field of retinal induction around a single horizontal bar of 20 mm. in length and 2 mm. in width was compared with the field set up by a horizontal broken bar of the same length and witdh. In this experiment the contrast effect at any point of the field was found greater for the broken bar than for the continuous one; the field around the continous bar extended over a distance of about 17 mm. in the direction perpendicular to the bar, while with the broken bar the corresponding distance was found to be about 31 mm. The greater extension of the field around the broken bar may be ascribed to its longer contour, but the possibility cannot be ruled out that other factors might be responsible for such a great difference of indirect induction caused by the continuous and the broken bars.

2. Summation of indirect induction

The arrangement of patches used in the following experiment is shown in Fig. 4. The contrast effect at the center of the half-ring was found to be 11, while the value at the center of the broken ring was as high as 27. The two inducing figures used here were the same in area, but somewhat different in the length of contour; the contour of the half-

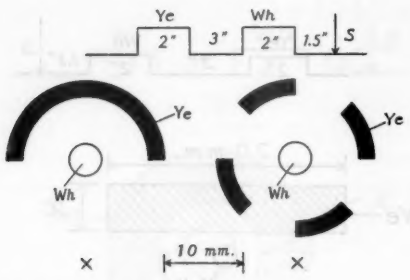

Fig. 4. All notations as in Fig. 2.

ring was 60.26 mm. while that of the broken ring was 72.26 mm. Although it cannot be denied that the contour is one of the most important factors for establishing indirect induction, the difference in the length of contour seems to be too small to account for the great difference in contrast effects in this case. It seems as if the high effectiveness of the broken ring is due to its configuration advantageous for summation of indirect induction. Generally, the value of indirect induction is found greater within a closed line figure than outside it. This fact shows that summation takes place more easily at a point enclosed by inducing sources. In the case of the half-ring the distribution of inducing sources is less enclosing than in the case of the broken ring, so that the summation of indirect induction must have accordingly been slight.

In order to study the effect of summation under simpler experimental conditions, the contrast effect was measured at a point midway between 2 vertical bars 20 mm. in length and 2 mm. in width and compared with the contrast effect obtained with a single bar. In Fig. 5 the values of contrast effects are represented as a function of distance from the margin of the bar. Though the area and contour of the double figure are twice as large as the single, the contrast effect in the former is not always twice as that in the latter; the ratio of the two values varies remarkably with the distance from the margin. These findings indicate that the indirect induction must be a very complicated function of distance, contour, configuration and other yet unknown factors.

3. Dependence of indirect induction on angles of inducing figures

The patch used in the following experiment is shown in the inset of Fig. 6. The effect of angle θ upon the contrast effect measured at point Wh was investigated as a function of θ. The contrast effect decreased as the angle θ increased and reached a minimum at 90°. It is apparent that the contour, area and distance remained almost unaltered in this experiment. In spite of this, the contrast effect greatly changed

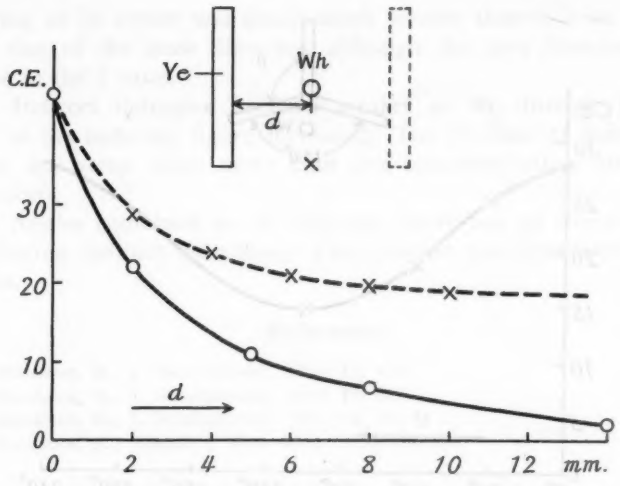

Fig. 5. Dependence of contrast effect (C.E.) on distance from margin of inducing yellow bar 20 mm. long and 2 mm. wide. Broken and continuous curves refer to induction caused by 2 bars and to that caused by a single bar respectively.

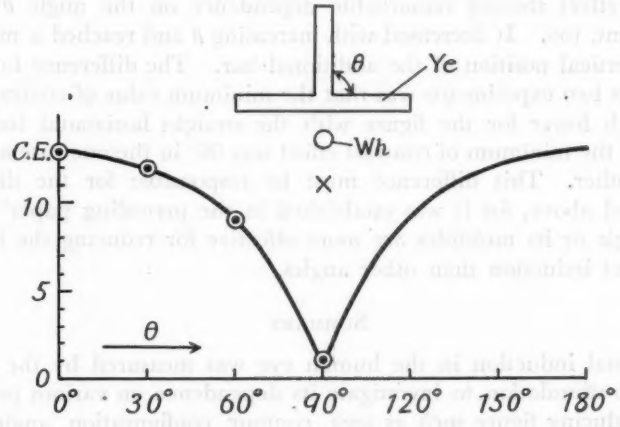

Fig. 6. Dependence of C.E. on angle.

depending on the angle θ. This fact indicates that the angle as such is an important factor to set up indirect induction.

Next, the inducing figure was so modified that the horizontal bar

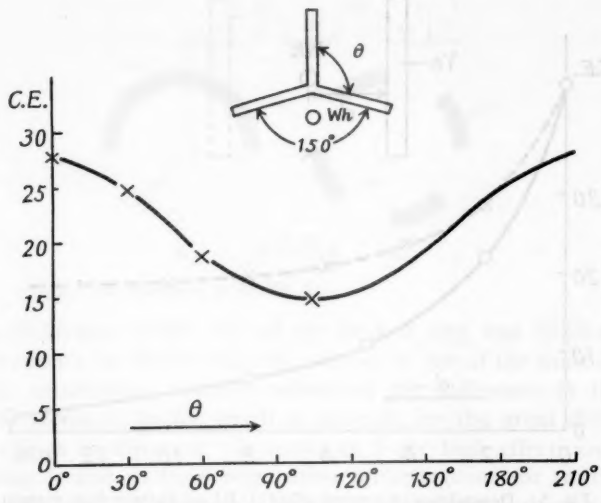

Fig. 7. Dependence of C.E. on angle.

was no more a straight line but consisting of 2 bars meeting at 150°, the other features being the same as in the experiment stated above. The contrast effect showed remarkable dependence on the angle θ in this experiment, too. It decreased with increasing θ and reached a minimum in the vertical position of the additional bar. The difference found between the two experiments was that the minimum value of contrast effect was much lower for the figure with the straight horizontal bar. The angle for the minimum of contrast effect was 90° in the one case and 105° in the other. This difference must be responsible for the difference mentioned above, for it was established in the preceding paper[2] that a right angle or its multiples are more effective for reducing the intensity of indirect induction than other angles.

Summary

Retinal induction in the human eye was measured by the method of electro-stimulation to investigate its dependence on various properties of the inducing figure such as area, contour, configuration, angle, etc.

1. Direct induction by which a physiological effect remaining within the area pre-illuminated is meant depends little on the area of the retinal image unless it is too small.

2. The same is true for indirect induction set up simultaneously around a retinal image; for example, the indirect induction set up by

a half-ring at its center was found much weaker than that set up by a broken ring of the same diameter, although the area illuminated was the same in the 2 cases.

3. Indirect induction becomes weaker as the distance from the margin of the inducing figure increases. The gradient of induction is, however, less steep when more than one inducing source are present (summation).

4. Angles contained in an inducing figure are an effective factor for producing indirect induction. This relation was illustrated by two examples.

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Studies on Blood Glycogen

The 3rd Report

The Blood Glycogen Content in Digestive Disease Patients

By

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Since Sanson[1] discovered in 1859 the glycogen in the blood, it has been generally recognized that glycogen is a constant ingredient in the blood, but it is not yet clear as to its function and significance.

According to our former experiments[2], the glycogen in the liver will be poured out into the blood as such, if the ability of the liver to keep glycogen is enfeebled, because the mobilization between glycogen and sugar takes place more rapidly and intensely than usual. Therefore, it is easily presumed that there is a close relation between the blood glycogen content and the liver function of patients suffering from digestive diseases.

Thus we attempted in the present experiments to clarify a clinical significance of the blood glycogen content in digestive disease patients.

Experimental

Method

For examination, the blood sample was taken from hungry patients early in the morning. The glycogen content in the blood was estimated by our own micromethod[3] and the blood sugar content by Fujita and Iwatake's method[4].

Results

1. *Blood glycogen content in healthy persons.* By our new micromethod we found in 10 healthy persons the highest and the lowest value to be 73 and 33 mg% respectively (the average value 53 mg%).

Therefore, 30–74 mg% is regarded as the normal range for the blood glycogen content (Table I).

2. *Gastric cancer.* The blood glycogen content obtained from 44

TABLE I

Blood Glycogen Content in Healthy Persons

Name	Sex	Age	Blood glycogen (mg%)	Blood sugar (mg%)
T.T.	♀	22	73.0	94
Y.R.	♂	17	73.0	65
S.C.	♀	21	72.0	110.5
T.H.	♂	24	66.0	88.0
M.M.	♂	23	65.5	84.0
S.T.	♂	28	64.5	84.5
Y.S.	♂	32	56.5	92.5
C.T.	♂	30	54.5	87.0
I.N.	♂	25	38.5	94.0
T.K.	♀	23	33.0	91.5

TABLE II

Blood Glycogen Content in Digestive Diseases

	Total	Decreased	Normal range	Elevated	Average
Healthy persons	10	0	10	0	53.0
Gastric cancer	44	3	29	12	63.5
Gastric and duodenal ulcers	28	2	22	4	55.5
Liver diseases	10	1	6	3	58.2
Diabetes mellitus	6	0	1	5	69.5

gastric cancer patients lies between 110.5 mg% and 16.5 mg% (the average value 63.5 mg%), which shows a higher value than the normal average value (Table II). Having divided them into two groups—the possible and the impossible of gastrectomy, 18 patients belonging to the former group showed 87–21 mg% (average 54 mg%) and 26 cases of the impossible group 110.5–16.5 mg% (average 63.5 mg%). Furthermore, according to the radiological classification of our Clinic[5], one case of Type 1 (polyplike cancer) showed 76 mg%, 18 cases of Type 2 (plateform or craterlike cancer) 110.5–21 mg% (average 65.8 mg%), 12 cases of Type 3 (scirrhus) 63–33 mg% (average 50 mg%) and 9 cases of Type 4 (mixed form) 101–16.5 mg% (average 59 mg%), as shown in Table III.

Therefore, it seems to be higher as the condition becomes worse and the influence upon the liver is more progressive.

3. *Gastric and duodenal ulcers.* 28 gastric and duodenal ulcer sufferers showed 89.5–21.5 mg% (average 55.5 mg%). In 17 cases of gastric ulcer we found only one case with a high value and in 9 cases of duodenal ulcer also one case with a high value (Table IV).

TABLE III

Blood Glycogen Content of Gastric Cancer

Classification	Operability	Decreased	Normal range	Elevated	Total
Type 1	Possible	0	0	1	1
	Impossible	0	0	0	0
	Total	0	0	1	1
Type 2	Possible	2	6	3	11
	Impossible	0	5	5	10
	Total	2	11	8	21
Type 3	Possible	0	6	0	6
	Impossible	0	4	0	4
	Total	0	10	0	10
Type 4	Possible	1	1	0	2
	Impossible	0	5	1	6
	Total	1	6	1	8
Total	Possible	3	13	4	20
	Impossible	0	16	8	24
	Total	3	29	12	44

TABLE IV

Blood Glycogen Content of Gastric and Duodenal Ulcers

	Decreased	Normal range	Elevated	Total
Gastric ulcer	1	13	3	17
Duodenal ulcer	1	7	1	9
Total	2	22	4	28

Consequently, there is no evident difference between gastric and duodenal ulcers.

4. *Liver diseases.* The average value of 10 liver patients (58.2 mg%), 5 cases of carcinoma of the liver and the bile duct, 2 cases of hepatitis, one case of Weil's disease, icterus simplex and cirrhosis, is a little higher than that of healthy persons (Table II). We found 3 cases (carcinoma of the liver and of the bile duct and icterus simplex) with a high value and only one case of carcinoma of the bile duct with a low value.

5. *Diabetes mellitus.* 5 cases out of 6 diabetes mellitus sufferers showed high values, though all fell between 114 and 47 mg%, the average being 69.5 mg%. One case with a normal value belonged to Type 1 (mild form), according to the classification of our Clinic[6] (Table V).

TABLE V

Blood Glycogen Content of Diabetes mellitus

	Decreased	Normal range	Elevated	Total
Type 1	0	1	2	3
Type 2	0	0	3	3
Total	0	1	5	6

DISCUSSION

The glycogen content in the blood in healthy persons is quite various under different methods. As a normal range, Eufinger[7] found 70 mg%, Schwarz and Pokrowskaja[8] 50 mg%, Gabbe[9] 18 to 30 mg%, Sikinami *et al*[10] 8.6 to 13.3 mg%, Golandas[11] 6.7 to 12.2 mg%, Kanda[12] 6.2 to 9.9 mg% and Unshelm[13] 5 to 8.6 mg%. According to our former report[3], it is clear that a lower concentration of alcohol is not sufficient for the complete precipitation of glycogen from the blood. As the results obtained by our new micromethod with a higher concentration of alcohol are 33 to 73 mg% (the average value 53 mg%), it is very natural that the normal range of the blood glycogen content should be from 30 to 74 mg%.

According to Staub and Golandas[14], the blood glycogen content of gastric cancer patients is higher than that of healthy persons. The results brought out from 44 gastric cancer sufferers by our own micromethod show a little higher value (the average value 63.5 mg%) than that of healthy persons, especially a higher value in the impossible cases of gastrectomy (the average value 63.5 mg%) than that of the possible group of gastrectomy (the average value 54 mg%). Furthermore, according to the radiological classification of our Clinic[5], the average value of Type 2 (65.8 mg%) is higher than that of other Types (Type 3, 50 mg%, Type 4, 59 mg%). Therefore, the value seems to be higher as the condition becomes worse and the influence upon the liver is more progressive.

The blood glycogen content of 28 gastric and duodenal ulcer patients is a little higher (average 55.5 mg%) than that of healthy persons (average 53 mg%), but there is no evident difference between gastric and duodenal ulcer cases.

Schwarz and Gerson[15] report that the more severely liver cells are destroyed, the more the glycogen content in the blood rises. The average value of 10 cases of liver diseases (58.2 mg%) is a little higher than that of healthy persons (53 mg%). Thus it is presumed that the blood glycogen content of severe liver patients is generally high.

Many investigators, such as Gabritschewsky[16], Schwarz and Gerson[15], Schwarz and Pokrowskaja[8], Okaya[17], Staub and Golandas[14], agree in

their opinion that the blood glycogen content of diabetes mellitus patients is in general elevated. Our results show that 5 cases out of 6 diabetes mellitus sufferers show high values, especially in the cases of Type 2, severe form, according to the classification of our Clinic[6].

Consequently, it can be concluded that an elevated glycogen content in the blood indicates a disturbed function of the liver also in the cases suffering from digestive diseases.

Summary

From these experiments on 88 digestive disease patients the following conclusion can be brought out:

1. The blood glyocgen content in 10 healthy persons lies between 33 and 73 mg% (the average value 53 mg%), therefore, 30–74 mg% is regarded as the normal range.

2. The blood glycogen contents brought out from 44 gastric cancer patients show a higher value (110.5–16.5 mg%, average 63.5 mg%) than the normal blood glycogen content, especially a higher value was given in the impossible cases of gastrectomy (average 63.5 mg%) than in the possible group of gastrectomy (average 54 mg%). Furthermore, according to the radiological classification of our Clinic, the average value of Type 2 (65.8 mg%) is higher than that of other Types (Type 3, 50 mg%, Type 4, 59 mg%).

3. The blood glycogen content of 28 gastric and duodenal ulcer sufferers is a little higher (89.5–21.5 mg%, average 55.5 mg%), but there is no evident difference between gastric and duodenal ulcer cases.

4. The blood glyocgen content of liver patients is also a little higher (82–27.5 mg%, average 58.2 mg%).

5. The blood glyocgen content of 6 cases of diabetes mellitus shows high values (114–47 mg%, average 69.5 mg%), especially in the cases of Type 2, severe form, according to the classification of our Clinic.

6. Consequently, it can be concluded that an elevated glycogen content in the blood indicates not only a disturbed function of the liver, but also an abnormal carbohydrate metabolism in the cases of digestive diseases.

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Studies on Blood and Plasma Catalase

The 1st Report

A New Micromethod of Estimation of Blood and Plasma Catalase

By

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Since Loew[1] in 1900 named "catalase" the substances releasing oxygen from hydrogen peroxide, there have appeared numerous publications regarding the catalase of the blood. At present there is no objection to the opinion that the catalase exists mainly in the blood-corpuscles. But workers differ in opinion as to the existence of the catalase in the plasma or serum. Many investigators, such as Becht[2], v. Thienen[3], Ochi[4] and Oppenheimer[5], deny its existence, but Kurokawa[6] and Takisawa[7] contend that it exists though it is very little.

Therefore, it is necessary to invent a micromethod, which makes it possible to perform the repeated estimations of the catalase content in the blood and plasma, in order to clarify the origin of the blood and plasma catalase and its deviation in various diseases.

From the standpoint mentioned above we made an attempt to estimate the catalase content in the 0.02 cc. of blood sample and the plasma catalase in the 0.1 cc. of blood sample.

Experimental

Method

1. Estimation of blood catalase

Put 0.02 cc. of the blood sample by means of an accurate pipette into a test-tube, which contains 20 cc. of destilled water, and mix them until a complete hemolysis is performed. Then put 1 cc. and 0.5 cc. of the solution separately into a test-tube, add 4 cc. of 0.3% hydrogen peroxide, whose pH is maintained at 7.0 with phosphate buffer of Sörensen. After catalyzing in a water tank at 38°C for 1 hour, add immediately 2 cc. of 20% sulphuric acid and 1 cc. of 25% potassium iodate, and then titrate the released iodine with 1/50 normal solution of sodium thiosulphate (x). As a blind test, furthermore, titrate the released iodine from 4 cc. of 0.3% hydrogen peroxide, 2 cc. of sulphuric acid and 1 cc. of 25%

potassium iodate, without the blood sample (X).

In the iodometry chemical reaction progresses as follows:

$$H_2O_2+2KJ+H_2SO_4=J_2+K_2SO_4+2H_2O$$
$$2Na_2S_2O_3+J_2=2NaJ+Na_2S_4O_6$$

Therefore, one molecule of hydrogen peroxide is appropriate to 2 molecules of sodium thiosulphate, hence, 1 cc. of 1/50 normal solution of the latter should be calculated as 1/100000 molecular weight of the former (one molecular weight is 34) and then 0.34 mg.

Thus the blood catalase (K) may be estimated from the following formula: $K=0.34\,(X\text{-}x)$.

K shows the content of hydrogen peroxide (g.), which is dissolved by 1 cc. of the blood sample at 38°C in 1 hour.

2. Estimation of plasma catalase

Put 0.1 cc. of the blood sample into the tube which is previously filled with 0.9 cc. of 3.5% sodium citrate solution, and centrifuge it for 3 minutes. 0.5 cc. of the supernatant liquid should be used for the estimation in the same manner as that of the blood catalase. The Plasma catalase content (K′) may be determined from the following formula: $K'=3.4\,(X\text{-}x)\times 2$.

K′ shows the content of hydrogen peroxide (mg.) which is dissolved by plasma from 1 cc. of the blood sample at 38°C in 1 hour.

Discussion

Following Inoue *et al.*[8], we adopted 38°C as the temperature of catalytic field and 7.0 as pH in the following experiments.

1. Duration of catalysis

Inoue *et al*[8] reported that either 30 minutes or 2 hours is the same for duration of catalysis, but Kamimura[9] recommended 90 minutes and Kurokawa[6] 40 minutes.

Table I shows that the catalytic power of the blood and plasma is the highest in 1 hour. Furthermore, according to Inoue[10], the catalytic process in the blood ends in the room temperature in 24 hours, but our results show that the catalytic process in the blood and plasma ends at 38°C within one hour.

This is the reason why the time to be used for catalysis is kept to one hour.

2. Spontaneous diminution of catalytic power

According to our report[11], the catalytic power of the thermal water diminishes more and more after gushing out. In the same manner, the catalytic power of the blood and plasma decreases after the lapse of time, and it can be reduced to one half after 2 hours (Table II).

Therefore, these experiments must be started as quickly as possible.

TABLE I
Duration of Catalysis

Name	Diagnosis	Catalase	Duration of catalysis (time in hours)			
			0.5	1	1.5	2
T.I.	Healthy	Blood	23.60	23.94	22.71	22.12
M.K.	ditto	ditto	21.35	19.99	19.86	18.70
N.E.	ditto	ditto	19.86	17.54	17.41	17.34
T.C.	Scirrhus	ditto	16.52	16.93		16.80
O.M.	Healthy	ditto	14.01	14.11	13.87	13.77
		Plasma	19.72	20.40	19.04	19.38
M.N.	Scirrhus	Blood	13.24	13.67		13.55
I.A.	Healthy	ditto	12.92	12.85	12.81	12.38
		Plasma	14.96	14.62	14.21	14.01
Rabbit No. 1	ditto	Blood	5.85	5.75	5.58	5.54
		Plasma	15.64	19.04	17.00	15.64
Rabbit No. 2	ditto	Blood	5.61	5.54	5.34	5.37
		Plasma	23.80	23.46	17.00	12.24

TABLE II
Spontaneous Diminution of Catalytic Power

Name	Diagnosis	Catalase	Before leaving alone	After leaving alone (time in hours)			
				0.5	1	1.5	2
T.I.	Healthy	Blood	23.94	18.22	17.27	18.63	12.65
M.K.	ditto	ditto	19.99	15.16	14.42	14.76	9.38
N.E.	ditto	ditto	17.55	14.48	14.55	14.69	9.04
O.M.	ditto	ditto	14.11	13.02	12.75	12.82	11.97
		Plasma	20.40	19.72	20.06	18.70	17.68
I.A.	ditto	Blood	12.85	12.21	11.97	11.83	11.59
		Plasma	14.62	14.08	14.11	14.01	13.84
Rabbit No. 1	ditto	Blood	6.02	5.10	5.17	4.66	4.66
		Plasma	18.70	15.64	15.64	17.00	10.20
Rabbit No. 2	ditto	Blood	5.95	5.41	5.44	5.27	4.83
		Plasma	24.14	23.80	23.12	23.80	22.44

3. Normal content of blood and plasma catalase in healthy persons

The blood catalase content in healthy persons is quite various under different methods, but it is generally accepted that the blood catalase content of the male is a little higher than that of the female (Nakanishi[12]). By our micromethod we found in 32 healthy persons the highest and the lowest value to be 25.80 and 9.80, respectively (the average value 17.79 $\pm$0.65). It is clear that the blood catalase content of the male (16 cases) is evidently higher than that of the female (16 cases), because the average value of the former is 19.73$\pm$0.93 and that of the latter 15.94$\pm$0.71 (Table III).

TABLE III

Normal Content of Blood Catalase in Healthy Persons

No.	Name	Sex	Age	Erythrocytes	Hb.	Hb-index	Blood Catalase	Catalase index	Hb-catalase index
1	S.Y.	♂	35	547	109	0.99	25.80	4.71	1.37
2	J.A.	♂	26	558	121	1.09	25.73	4.60	1.24
3	S.A.	♂	27	577	106	0.92	24.14	4.18	1.32
4	T.S.	♂	25	545.3	125	1.15	24.04	4.41	1.12
5	T.H.	♂	31	506.5	109	1.08	23.22	4.58	1.24
6	S.S.	♀	25	534	126	1.18	22.64	4.25	1.04
7	N.A.	♂	26	562	119	1.06	22.37	3.98	1.09
8	T.N.	♂	31	517.5	109	1.05	22.27	4.31	1.19
9	G.I.	♂	33	499.5	102	1.02	22.27	4.46	1.27
10	T.A.	♂	35	536	131	1.22	21.08	3.92	0.93
11	Z.A.	♂	25	516	109	1.05	20.94	4.06	1.12
12	S.Y.	♂	31	519	102	0.98	20.81	4.01	1.19
13	Y.S.	♀	23	473	114	1.20	20.81	4.40	1.06
14	Z.K.	♂	26	463	92.5	0.99	20.60	4.44	1.30
15	M.A.	♂	24	444.5	107.5	1.21	20.54	4.62	1.11
16	M.K.	♀	24	473	120	1.27	20.13	4.28	0.97
17	R.O.	♀	23	533.5	114	1.07	19.31	3.62	0.99
18	Y.A.	♂	26	520	117	1.12	19.18	3.69	0.95
19	K.W.	♀	25	488	110	1.13	18.94	3.88	1.00
20	M.U.	♀	21	479.5	101	1.05	18.90	3.95	1.09
21	K.H.	♀	23	493.5	117	1.19	18.87	3.83	0.94
22	F.C.	♀	25	417.5	107.5	1.29	17.61	4.22	0.95
23	O.M.	♀	26	591	121	1.01	17.17	2.90	0.83
24	I.T.	♀	20	434.5	114	1.32	16.59	3.82	0.85
25	M.E.	♀	24	452	110	1.22	16.12	3.57	0.85
26	M.T.	♀	24	472.3	81	0.86	16.05	3.39	1.15
27	T.N.	♂	29	469.5	102	1.09	15.84	3.88	0.90
28	M.S.	♀	26	435	107	1.23	15.23	3.52	0.83
29	T.N.	♀	24	436	114	1.37	14.93	3.42	0.76
30	T.T.	♀	18	449	108	1.11	13.36	2.98	0.72
31	H.K.	♀	25	513	123	1.20	12.89	2.50	0.61
32	R.T.	♀	23	481	114	1.18	11.32	2.36	0.58

According to Kurokawa[6] and Takisawa[7], the plasma catalase content is very little. The results obtained by our micromethod show that the plasma catalase content lies from 17.00 to 10.20, the average being 14.10 $\pm$0.67, and there is no clear difference between the male and the female, because the average value of the former is 13.90$\pm$0.93 and that of the

TABLE IV

Normal Content of Plasma Catalase in Healthy Persons

No.	Name	Sex	Age	Breakfast	Erythrocytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-Blood catalase ratio
1	R.K.	♂	40	Before	526	122	1.16	10.64	17.00	2.12	0.51	1.60
				1h after	531	120	1.13	10.74	16.66	2.02	0.52	1.55
2	I.E.	♀	23	Before	493	82	0.83	13.29	16.32	2.70	0.94	1.23
				2h after	502	83	0.83	14.21	15.64	2.83	0.99	1.10
3	Y.T.	♀	23	Before	459	81	0.88	9.79	15.64	2.13	0.70	1.60
				1h after	463	88	0.95	9.86	16.66	2.13	0.65	1.69
4	A.I.	♀	21	Before	482	96	1.00	12.44	15.64	2.57	0.74	1.26
				2h after	516	103	1.00	14.01	14.62	2.71	0.79	1.04
5	M.O.	♂	25	Before	528	108	1.02	15.98	14.28	3.03	0.86	0.89
				3h after	519	99	0.95	14.96	14.62	2.98	0.88	0.98
6	S.S.	♂	33	Before	456	89	0.98	12.24	13.60	2.69	0.80	1.11
				1h after	463	86	0.93	12.31	14.28	2.66	0.83	1.16
7	S.T.	♀	22	Before	486	103	1.06	12.44	13.60	2.55	0.69	1.09
				2h after	499	101	1.01	12.38	14.28	2.47	0.71	1.15
8	Z.A.	♂	25	Before	508	110	1.08	15.30	12.92	3.01	0.81	0.85
				3h after	534	118	1.10	14.28	13.26	2.48	0.70	0.93
9	T.S.	♂	24	Before	520	115	1.10	11.16	11.56	2.15	0.56	1.04
				1h after	523	121	1.15	11.22	12.24	2.15	0.54	1.09
10	S.S.	♀	26	Before	514	101	0.98	10.09	10.20	1.96	0.58	1.01
				1h after	511	98	0.96	10.03	10.54	1.95	0.61	1.02

latter 14.30±0.97 (Table IV).

Moreover, the plasma-blood catalase ratio in healthy persons which is the quotient of the plasma catalase content divided by that of the blood, falls from 1.60 to 0.89, the average being 1.18±0.085 (Table IV).

At present it is generally recognized that a large part of the blood catalase exists in erythrocytes. Therefore, v.Thienen[8] named the quotient of the blood catalase (g.) to erythrocytes (million) a catalase index. Furthermore, Nakanishi[12] gave a name hemoglobin-catalase index to the quotient of the blood catalase (g.) to hemoglobin (mg.). After our micromethod we found the catalase index in healthy persons, the higheset and the lowest value being 4.71 and 1.91, and the average 3.62±0.117. The catalase index of the male is a little higher than that of the female, the average value of the former being 3.89±0.155 and that of the latter 3.36± 0.160. Moreover, our results show that the hemoglobin-catalase index falls between 1.37 and 0.51, the average being 0.96±0.033, and that of the male (average 1.06±0.048) is higher than that of the female (average 0.86±0.034). By the by, we count in these experiments 100% of the hemoglobin content by Sahli's method as 17.2 mg after Bürker[13].

4. Normal content of blood and plasma catalase in normal rabbits

It is reported by Lesser[14] and Watanabe[15] that the blood catalase content differs with the species of animal. The results of 80 experiments with 20 rabbits show that the blood catalase content falls, as shown in Table V, from 11.39 to 3.06 and the average value (6.38±0.62) is evidently lower than that of healthy men (17.79±0.65). On the contrary, the plasma catalase content of rabbits lies between 43.52 and 8.16 and the average value (23.03±1.06) is higher than that of healthy men (14.10 ±0.67). Therefore, the plasma-blood catalase ratio in normal rabbits falls from 8.02 to 0.95, the average being 4.02±0.248, and it is evidently higher than that of men. Moreover, the catalase index of rabbits lies between 1.78 and 0.57, the average being 1.05±0.037 and those hemoglobin-catalase index between 0.78 and 0.28, the average 0.44±0.013. Therefore, those results from rabbits are distinctly higher than that of men.

5. Physiological variation of blood and plasma catalase

As to a dietary influence upon the blood catalase content Moraczewski[16] and Magat[17] noticed its elevation, but Alexeeff[18] and Shindo[19] asserted it as uninfluential. Furthermore, according to Kurokawa[6] the plasma catalase is not influenced by diet. The results presented in Table IV, V and VI show that a dietary or diurnal influence upon the blood and plasma catalase content of healthy persons and rabbits lies almost within the limits of physiological variation.

TABLE V

Normal Content of Blood and Plasma Catalase in Normal Rabbits

No.	Sex	Datum	Erythrocytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-Blood catalase ratio
1	♂	1st day	532	71	0.67	9.52	26.52	1.79	0.78	2.79
		2nd "	551	73	0.66	7.31	24.48	1.32	0.58	3.35
2	♀	1st "	617	97	0.79	8.87	27.88	1.44	0.53	3.14
		2nd "	630	94	0.75	9.35	36.04	1.48	0.58	3.85
3	♂	1st "	549	75	0.68	8.16	35.86	1.37	0.63	4.32
		3rd "	483	68	0.70	6.24	35.36	1.29	0.53	5.66
4	♀	1st "	622	87	0.70	7.72	8.84	1.28	0.51	1.14
		2nd "	610	88	0.72	7.21	10.88	1.18	0.47	1.51
5	♀	1st "	585	88	0.75	7.68	12.24	1.31	0.51	1.59
		3rd "	631	94	0.74	8.33	14.28	1.32	0.51	1.71
6	♀	1st "	607	90	0.74	7.58	23.12	1.25	0.49	3.05
		8th "	641	103	0.81	11.39	14.96	1.78	0.64	1.31
		20th "	600	93	0.78	8.74	10.88	1.45	0.55	1.25
7	♀	1st "	599	79	0.66	7.41	17.68	1.24	0.54	2.39
		2nd "	593	83	0.70	7.75	12.24	1.31	0.54	1.57
8	♀	1st "	695	98	0.70	7.41	11.56	1.06	0.44	1.56
		2nd "	697	99	0.72	7.41	8.16	1.06	0.44	1.10
9	♀	1st "	745	107	0.72	6.94	13.60	0.93	0.37	1.90
		3rd "	700	98	0.70	5.61	14.96	0.80	0.33	2.67
10	♂	1st "	586	86	0.73	6.49	17.00	1.11	0.44	2.62
		3rd "	556	82	0.74	5.68	12.24	1.02	0.40	2.16
11	♀	1st "	676	80	0.58	6.22	32.64	1.28	0.45	5.25
		2nd "	681	85	0.63	5.47	27.20	0.81	0.38	4.97
12	♀	1st "	652	90	0.69	5.81	33.66	0.89	0.38	5.79
		4th "	608	84	0.69	4.62	19.72	0.76	0.32	4.26
13	♀	1st "	599	80	0.67	5.78	40.80	0.96	0.42	7.06
		2nd "	639	80	0.63	5.58	36.04	0.82	0.40	6.46
14	♀	1st "	631	82	0.65	5.75	25.50	0.91	0.41	4.43
		5th "	642	67	0.57	5.37	29.24	0.84	0.47	5.18
15	♀	1st "	626	87	0.69	5.34	26.52	0.86	0.36	4.97
		6th "	598	77	0.64	5.07	20.40	0.85	0.38	4.03
		16th "	575	82	0.71	5.51	23.80	0.96	0.39	4.32
16	♂	1st "	750	103	0.69	5.34	23.80	0.71	0.31	4.46
		4th "	671	100	0.75	4.62	15.98	0.69	0.27	3.46
17	♂	1st "	639	85	0.66	5.03	37.40	0.79	0.34	7.47
		6th "	683	96	0.69	4.96	34.00	0.73	0.30	6.84
18	♀	1st "	632	90	0.71	4.93	14.28	0.78	0.32	2.89
		12th "	597	88	0.74	4.79	10.20	0.80	0.32	2.13
19	♂	1st "	575	76	0.66	4.52	34.00	0.79	0.35	7.52
		3rd "	584	78	0.67	5.00	29.24	0.85	0.37	5.87
20	♀	1st "	532	66	0.62	3.06	24.48	0.58	0.27	8.02
		4th "	550	70	0.64	3.40	27.20	0.62	0.28	8.01

TABLE VI

Influence of Diet upon Blood Catalase

Name	Before breakfast	After breakfast	Before dinner	Before supper
S.Y. (1st day)	20.80	21.93	19.96	
(2nd ")	21.93			21.49
T.N. (1st ")	15.84	17.78	16.35	
(2nd ")	17.14			17.34
H.C.	17.61	20.23	18.33	
I.T.	16.59	15.10		
M.S.	15.23	13.67		
S.S.	20.81			21.56

SUMMARY

Our experiments present a new micromethod which makes it possible to perform the accurate estimation of the blood catalase in the 0.02 cc. of blood sample and that of the plasma catalase in the 0.1 cc. of blood sample, and we report the normal content of the blood and plasma catalase in men and rabbits.

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Studies on Blood and Plasma Catalase

The 2nd Report

The Blood Catalase Content in Digestive Disease Patients

By

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At present, workers differ in opinion as to the function and significance of the catalase in the blood. But many investigators, such as Jolles[1], Lesser[2], Ewald[3], Ostwald[4], Burge[5], Iizuka and Watanabe[6], Tomioka[7] and Kita[8], have asserted that the catalase concerns practically with the oxidation in a living body. Nevertheless, the clinical significance of the blood catalase is not yet clear.

Therefore, we attempted to estimate the blood catalase by the same method as in the first report[9] and clarify its diagnostic and prognostic value in digestive disease patients.

Experimental

Results

1. Blood catalase content in healthy persons

Under our micromethod, we found 35 estimations in 32 healthy persons the highest and the lowest value to be 25.80 and 9.79 respectively, the average value being 17.79 ± 0.65 (Table I). And then the catalase index lies between 4.71 and 1.96, the average being 3.62 ± 0.117 and the hemoglobin-catalase index between 1.37 and 0.51, the average being 0.96 ± 0.033.

2. Gastric and duodenal ulcers

The blood catalase content obtained from 192 estimations of 100 ulcer sufferers, 62 gastric ulcers, 31 duodenal ulcers and 7 gastroduodenal ulcers, lies between 24.24 and 6.32, the average being 14.55 ± 0.34, showing clearly a lower value than the normal average value (Table I). Moreover, the catalase index and hemoglobin-catalase index in cases of ulcer patients tend to decrease compared with healthy persons, because the former falls from 4.87 to 1.69, the average being 3.38 ± 0.06 and the latter

TABLE I

Blood Catalase of Various Diseases

Diagnosis	Total	Blood catalase			Catalase index			Hb- catalase index		
		Highest value	Lowest value	Average value	Highest value	Lowest value	Average value	Highest value	Lowest value	Average value
Healthy	35	25.80	9.79	17.79 ±0.65	4.71	1.96	3.62 ±0.117	1.37	0.51	0.96 ±0.033
Ulcers	192	24.24	6.32	14.55 ±0.34	4.87	1.69	3.38 ±0.06	1.45	0.42	0.87 ±0.015
Gastric cancer	70	23.80	4.73	12.17 ±0.55	4.57	1.66	3.06 ±0.09	1.63	0.41	0.81 ±0.023
Other cancers	19	21.52	7.41	13.87 ±1.11	5.95	2.38	3.45 ±0.20	1.24	0.52	0.78 ±0.050
Other diseases	48	21.69	2.96	12.85 ±0.68	5.40	1.49	3.29 ±0.14	1.34	0.34	0.78 ±0.031

TABLE II

Blood Catalase of Gastric and Duodenal Ulcers

Diagnosis	Total	Blood catalase	Catalase index	Hemoglobin-catalase index
Gastric ulcer	126	14.43±0.36	3.40±0.08	0.85±0.07
Duodenal ulcer	49	15.39±0.63	3.42±0.10	0.91±0.03

from 1.45 to 0.42, the average being 0.87±0.015. Table II shows that there is no evident difference between gastric and duodenal ulcers as regards the blood catalase, catalase index and hemoglobin-catalase index.

As to the relation of the progress of ulcers with the blood catalase content, we found from 50 ulcer patients that the blood catalase content increases for the most part when ulcers are cured, especially in severe cases of the incipient stage (Table III). Under these circumstances the catalase index and hemoglobin-catalase index have a tendency to elevate with the increase of the blood catalase content.

Therefore, it is presumed that the increase of the catalase content in the blood precedes the recovery of erythrocytes and hemoglobin in ulcer cases.

3. Gastric cancer

The blood catalase content obtained from 70 measurements of 49 gastric cancer patients shows the highest and the lowest value to be 23.80 and 4.73, the average being 12.17±0.55, showing an evident lower value than that of healthy persons and ucler sufferers. Moreover, the average value of the catalase index (3.06±0.09) and hemoglobin-catalase index (0.81±0.023) in the cases of gastric cancer are also remarkably lower

TABLE III

Progress of Ulcers and Blood Catalase

No.	Name	Diagnosis	Date	Blood catalase	Catalase index	Hb-catalase index	Erythrocytes	Hb.	Hb-index
1	K.S.	Gastric ulcer	21/VIII	6.60	2.00	0.70	330	55.2	1.07
			7/IX	5.95	2.04	0.51	291	67.5	1.16
			21/IX	7.92	2.02	0.52	391.5	87.5	1.12
			5/X	10.61	2.54	0.70	417.5	87.5	1.05
2	O.K.	ditto	11/IX	9.55	2.71	0.68	352	81.4	1.15
			29/IX	11.46	2.79	0.74	410	90	1.11
			19/X	13.29	3.72	0.68	354	114	1.61
			10/XI	12.61	3.34	0.67	379.5	110	1.45
34	T.Y.	ditto	4/IX	12.21	3.35	0.66	364	107	1.47
			21/IX	9.86	2.41	0.59	409.5	97.5	1.19
			2/X	8.26	1.98	0.51	416	94	1.13
35	S.G.	Duodenal ulcer	5/XI	6.32	3.61	0.74	175	50	1.43
			19/XI	10.68	3.89	0.88	244.5	70	1.28
			4/XII	14.96	3.81	1.04	391	83.8	1.07
37	O.M.	ditto	21/VIII	9.55	2.29	0.80	418	68.8	0.66
			7/IX	11.73	2.35	0.70	499	97	0.99
			22/IX	12.65	2.55	0.77	496	96.0	0.96
43	M.T.	ditto	7/VIII	13.91	2.52	0.65	553	125	1.13
			28/VIII	11.46	2.16	0.76	367	88	1.20
			14/IX	10.37	2.39	0.60	433	101	1.17

than that of healthy persons and ulcer patients (Table I). Having divided them into two groups according to the possibility of gastrectomy, the average value of the blood catalase content, catalase index and hemoglobin-catalase index from 23 patients belonging to the possible group are respectively 13.32, 3.14 and 0.82, and tend to be more elevated than 24 cases of the impossible group, of which the average value of the blood catalase, catalase index and hemoglobin-catalase index are respectively 11.45, 3.06 and 0.85 (Table IV). Such a tendency is more remarkable in the case of Type 3 (scirrhus) than Type 2 (plateform or craterlike cancer), according to the radiological classification of our Clinic[10], especially the average value of Type 4 (mixed form), which is diagnosed in impossible stage of gastrectomy, is the lowest, as is shown in Table V.

Furthermore, as to the relation of the progress of gastric cancer with the blood catalase content, the latter generally decreases in the impossible group of gastrectomy, when the former is more malignant (Table V).

Thus the estimation of the blood catalase content is available in the determination of gastrectomy to a certain extent.

4. Other digestive diseases

The results obtained from 19 estimations of 9 various cancers of diges-

TABLE IV

Possibility of Gastric Cancer for Gastrectomy and Blood Catalase

Form	The Possible group			The Impossible group			Total		
	Blood catalase	Catalase index	Hb-catalase index	Blood catalase	Catalase index	Hb-catalase index	Blood catalase	Catalase index	Hb-catalase index
Type 2	12.36 ±1.13	3.25 ±0.27	0.78 ±0.066	11.88 ±1.38	3.25 ±0.26	0.91 ±0.088	12.13 ±0.88	3.25 ±0.15	0.86 ±0.050
Type 3	13.93 ±1.32	3.07 ±0.18	0.85 ±0.058	11.25 ±1.35	3.13 ±0.30	0.85 ±0.088	13.46 ±0.95	3.10 ±0.16	0.85 ±0.050
Type 4				10.00 ±2.90	2.50 ±0.52	0.73 ±0.105	10.00 ±2.90	2.50 ±0.52	0.73 ±0.105
Total	13.32 ±0.93	3.14 ±0.14	0.82 ±0.043	11.45 ±0.96	3.06 ±0.20	0.85 ±0.059	12.10 ±0.67	3.10 ±0.12	0.84 ±0.036

TABLE V

Progress of Gastric Cancer and Blood Catalase

No.	Name	Diagnosis	Date	Blood catalase	Catalase index	Hb-catalase index	Erythro-cytes	Hb.	Hb-index
1	S.S.	Cancer of cardia (imposs.)	10/VIII	10.44	2.24	0.61	464.5	100	1.08
			1/IX	8.13	1.78	0.44	456.	108	1.20
			21/IX	8.81	2.32	0.53	376.5	95	1.26
2	S.T.	Type 2 (imposs.)	28/IX	14.38	4.64	0.93	316	90	1.45
			13/X	11.87	2.90	0.71	409.5	97.5	1.19
4	T.M.	Type 2 (possib.)	3/XI	8.60	3.04	0.73	279	68.6	1.23
			10/XI	12.51	4.10	0.91	300.5	80	1.33
6	W.H.	Type 3 (possib.)	7/IX	6.94	1.79	0.61	383.5	66.3	0.87
			21/IX	8.09	2.44	0.69	331.5	62.5	0.94
			2/X	8.57	2.52	0.61	339	81	1.19
8	H.I.	Type 4 (imposs.)	7/VIII	4.73	1.94	0.48	243	57.2	1.17
			21/VIII	5.54	1.98	0.53	279.5	61.5	1.10
			28/VIII	8.74	2.72	0.62	321.5	81.5	1.26

tive diseases show a lower value than that of healthy persons (Table I), of which 3 cases of cancer of the oesophagus present almost a normal value, 3 cases of cancer of the liver a little lower value and 3 cases of cancer of the bile duct an evident lower value (Table VI).

Other liver diseases, including 5 cases of cirrhosis of the liver and of cholelithiasis, show a lower value than that of healthy persons. But the blood catalase content of diabetes mellitus presents almost a normal value (Table VI).

Generally speaking, therefore, the catalase content in the blood tends to decrease in the cases of various cancers and severe form of the liver diseases.

5. Relation between blood catalase, erythrocytes and hemoglobin

TABLE VI
Blood Catalase of Other Digestive Diseases

Diagnosis	Blood catalase			Catalase index			Hb-catalase index		
	Highest value	Lowest value	Average value	Highest value	Lowest value	Average value	Highest value	Lowest value	Average value
Cancer of oesophagus	21.52	11.56	16.00 ±2.49	4.64	2.73	3.50 ±0.94	1.24	0.78	0.98 ±0.115
Cancer of liver	18.67	9.52	14.00 ±1.89	5.95	3.90	4.50 ±0.94	1.14	0.82	0.98 ±0.057
Cancer of bile duct	9.01	7.41	8.67 ±1.03	2.99	2.38	2.50 ±0.11	0.71	0.56	0.65 ±0.039
Cirrhosis of liver	17.85	2.96	10.80 ±2.44	3.72	2.59	3.20 ±0.36	0.98	0.77	0.94 ±0.049
Cholelithiasis	19.92	7.11	12.80 ±2.00	5.40	1.80	2.90 ±1.34	1.08	0.43	0.78 ±0.238
Diabetes mellitus	19.02	5.04	16.00 ±1.60	5.17	1.65	3.50 ±1.26	1.10	0.42	0.85 ±0.070

TABLE VII
Coefficient of Correlation

	Erythrocytes	Hb.	Hb-index	Sedimentation rate	Rohrer's index
Blood catalase	+0.628 ±0.038	+0.489 ±0.046	−0.093 ±0.052	−0.387 ±0.059	−0.008 ±0.065
Catalase index		+0.442 ±0.042	+0.168 ±0.051	−0.160 ±0.007	−0.023 ±0.018
Hb-catalase index	+0.347 ±0.046		−0.353 ±0.046	−0.072 ±0.068	−0.042 ±0.061

From 364 meausrements as regards the relation between blood catalase, erythroctyes and hemoglobin, we have calculated the coefficient of correlation as is shown in Table VII, and concluded that there is a positive correlation between blood catalase and erythrocytes, blood catalase and hemoglobin, catalase index and hemoglobin, catalase index and hemoglobin index, erythrocytes and hemoglobin-catalase index, and a negative correlation between blood catalase and sedimentation rate, catalase index and sedimentation rate, hemoglobin-catalase index and hemoglobin index.

These results suggest a close relation of the blood catalase with erythrocytes and hemoglobin.

DISCUSSION

As for the blood catalase content of gastric and duodenal ulcers, Strauss and Rammelt[11] asserted its decrease in a small number of ulcer

patient, but Segall and Händel[12] and Nakanishi[13] reported it as unchangeable. The results obtained from 192 estimations of 100 ulcer cases show that the blood catalase content, catalase index and hemoglobin-catalase index tend to decrease, compared with healthy persons, but there is no evident difference between gastric and duodenal ulcers, and increase when ulcers are cured, preceding the recovery of erythrocytes and hemoglobin.

It is generally recogized that the blood catalase content decreases in the cases of various cancers (Jolles and Oppenheim,[14] Brahn and Hirschfeld[15], v. Thienen[16], Zerner[17], Nakanishi[13], Shindo[18], Deutsch and Fränkel[19]). But investigators differ in opinion as to the catalase index of cancer patients. According to v. Thienen[16], Zerner[17] and Nakanishi[13], the catalase index is not changeable, while Shindo[18] asserts its increase and Deutsch and Fränkel[19] believe its decrease. The results obtained from 70 measurements of 49 cases of gastric cancer show an evident lower value than healthy persons and ulcer patients, and such a tendency is more remarkable in the cases of the impossible group of gastrectomy than in the cases of the possible ones, especially in the cases of Type 3 (scirrhus) than Type 2 (plateform or craterlike cancer), according to the radiological classification of our Clinic.[10] Moreover, the results brought out from 9 gastric cancer patients generally decrease in the impossible group, when their process is more malignant. Furthermore, generally speaking, the catalase content in the blood tends to decrease in the cases of other various cancers and severe form of the liver diseases.

It is clear that a large portion of the catalase exists in red corpuscles (Jolles[1], Jolles and Oppenheim[14], Loeb and Mulzer[20], Becht[21], Ochi[22]). The coefficient of correlation calculated from 364 measurements shows that there are the positive correlations between blood catalase and erythrocytes as well as hemoglobin, between catalase index and hemoglobin as well as hemoglobin index, and between hemoglobin-catalase index and erythrocytes, but negative correlations between sedimentation rate and either of blood catalase and catalase index, as for hemoglobin-catalase index and hemoglobin index. These results suggest the close relation of the blood catalase with erythrocytes and hemoglobin.

Therefore, it is concluded that the estimation of the blood catalase content is available for the determination of prognosis of various digestive diseases to a certain extent.

Summary

From our experiments we had the following results: the blood catalase content changes for itself in spite of the close connection with erythrocytes and hemoglobin and it is available in the determination of prognosis of

various digestive disease patients to a certain extent.

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